UNIVERSIDAD AUTÓNOMA DE MADRID

FACULTAD DE MEDICINA

RELACIÓN ENTRE LA MORFOLOGÍA Y LA FUNCIÓN PERITONEAL EN PACIENTES TRATADOS CON DIÁLISIS PERITONEAL CON SOLUCIONES CONVENCIONALES. ANÁLISIS DEL IMPACTO QUE TIENEN LA DURACIÓN DE LA TÉCNICA Y EL USO DE SOLUCIONES MÁS BIOCOMPATIBLES

TESIS DOCTORAL

Gloria del Peso Gilsanz

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La presentación de la Tesis Doctoral titulada: "Relación entre la morfología y la función peritoneal en pacientes tratados con diálisis peritoneal con soluciones convencionales. Análisis del impacto que tienen la duración de la técnica y el uso de soluciones más biocompatibles", realizada por Dña. GLORIA DEL PESO GILSANZ, bajo mi dirección y supervisión, y que presenta para la obtención del grado de Doctora por la Universidad Autónoma de Madrid.

En Madrid, a 6 de junio de 2014

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A mis padres

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Memoria presentada para optar al grado de Doctora en Medicina por la Universidad Autónoma de Madrid

GLORIA DEL PESO GILSANZ

ÍNDICE

1.- ABREVIATURAS

AGEs:	productos de glicosilación avanzada
α-SMA:	actina de músculo liso
AT:	alto transporte
DP:	diálisis peritoneal
DPA:	diálisis peritoneal automática
DPCA:	diálisis peritoneal continua ambulatoria
D/P-Cr:	cociente dializado/plasma de creatinina
FGF:	factor de crecimiento fibroblástico
FRR:	función renal residual
ml:	mililitros
MTC:	coeficiente de transferencia de masas
MTC-Cr:	coeficiente de transferencia de masas de creatinina
MTC-U:	coeficiente de transferencia de masas de urea
PCR:	proteína C reactiva
PDGs:	productos de degradación de la glucosa
PET:	test de equilibrio peritoneal
TEM:	transición epitelio mesenquimal
TGF-β:	factor de crecimiento transformante
UF:	ultrafiltración
VEGF:	factor de crecimiento del endotelio vascular
VH:	vasculopatía hialinizante

2.- INTRODUCCIÓN

2.1.- La diálisis peritoneal

2.1.1.- Conceptos

La diálisis peritoneal (DP) es una técnica sustitutiva renal utilizada desde hace años, que se basa en el uso del peritoneo como una membrana semipermeable, a través de la cual se realiza intercambio de agua (transporte convectivo) y de solutos (transporte difusivo). Este intercambio se produce entre la sangre de los capilares peritoneales y un líquido de diálisis hiperosmolar que se introduce en la cavidad abdominal. Uno de los principales objetivos de la DP es la preservación de la membrana peritoneal a largo plazo, por ello, su principal limitación es la incapacidad del peritoneo para realizar un adecuado transporte convectivo y difusivo durante largos periodos (1,2,3,4). El fallo del transporte de agua o fallo de ultrafiltración (UF) es la alteración funcional más frecuente de la membrana peritoneal (5), siendo en parte responsable de la sobrecarga de volumen y el incremento del riesgo cardiovascular que presentan estos pacientes (6,7). La sobrecarga de volumen es un factor de riesgo independiente de mortalidad en pacientes en DP (8). El fallo de UF es además, junto con las infecciones peritoneales, la causa más frecuente de salida de la técnica. Los principales factores que se han relacionado con su aparición son la exposición prolongada a soluciones bioincompatibles (con alto contenido en glucosa y derivados, hiperosmóticas y con bajo pH), así como los episodios severos o repetidos de peritonitis y los hemoperitoneos.

2.1.2.- Soluciones de diálisis

Las soluciones de DP están compuestas de agua, electrolitos, un agente osmótico y un tampón. Los principales factores asociados con su bioincompatibilidad son el pH ácido, la elevada osmolaridad, el uso de lactato como tampón, y el alto contenido en glucosa y en productos de degradación de la glucosa (PDGs)(que se forman durante la esterilización de las soluciones)(9,10).

La eliminación de agua requiere la existencia de un gradiente de presión osmótica entre la sangre y la solución de diálisis. Para ello, las soluciones de DP contienen un agente osmótico, de los cuales el más utilizado habitualmente es la glucosa. Las soluciones glucosadas denominadas **convencionales** tienen bolsas monocamerales, pH ácido, elevada osmolaridad, utilizan lactato como tampón, y son ricas en glucosa y PDGs. Por el contrario, las soluciones más **biocompatibles** utilizan sistemas con doble o triple bolsa, tienen un pH más fisiológico, emplean como tampón bicarbonato, lactato o una mezcla de ambos, y contienen bajos niveles de PDGs (11). Los beneficios teóricos del uso de bolsas multicompartimentales más biocompatibles han sido demostrados en estudios *in vitro* y *ex vivo*, así como en modelos animales experimentales (12,13). Inducen mayor conservación de la integridad mesotelial (14), menor acúmulo de productos de glicosilación avanzada (AGEs) y mejoran los mecanismos de defensa peritoneal (15). En animales, se ha observado aumento de la capacidad de UF y menor inducción de fibrosis y angiogénesis (16). En humanos sus efectos son más controvertidos, habiéndose descrito en algunos ensayos clínicos controlados (17,18) mejor control de la acidosis metabólica, mejor preservación de la función renal residual y retraso de la aparición de anuria. Los resultados sobre supervivencia del paciente y la

técnica son aún poco conocidos, y los estudios sobre incidencia de peritonitis son hasta el momento contradictorios (17,18,19).

Actualmente, solo existen dos soluciones que utilicen agentes osmóticos distintos a la glucosa: la icodextrina al 7.5% y los aminoácidos al 1.1%. Ambas utilizan lactato como tampón. La icodextrina es un polímero de glucosa que se absorbe mucho más lentamente que la glucosa, por lo que mantiene más tiempo la presión oncótica, e induce una UF más duradera en el tiempo que ésta y equivalente a la obtenida con glucosa al 3.86%. Es especialmente eficaz durante episodios de peritonitis y en pacientes con fallo de UF. Por otro lado, las soluciones de aminoácidos contienen una mezcla de aminoácidos esenciales y no esenciales, su poder oncótico es similar al de la glucosa al 1.36% y pueden ser utilizados con fines nutricionales en pacientes desnutridos (20).

2.2.- Anatomía peritoneal

En el ámbito de la DP, el estudio de la biopsia peritoneal nos puede permitir conocer el sustrato morfológico de las alteraciones funcionales peritoneales presentes en muchos pacientes, pero además puede ayudarnos a explorar los mecanismos fisiopatológicos responsables de estos cambios. La invasividad de la técnica provoca que sean muy escasos los estudios que incluyen biopsia peritoneal. No hay un método estandarizado para el procesamiento de las muestras peritoneales (habitualmente realizado mediante fijación en formol e inclusión en parafina) ni sobre cuáles métodos cuantitativos y semicuantitativos deben ser utilizados para la mediciones histológicas. Por

ello, hay que ser prudentes a la hora de comparar los distintos estudios morfológicos.

2.2.1.- En sujetos sanos (Figura 1)

La membrana peritoneal normal (Figura 1) se compone de las siguientes estructuras:

A. Mesotelio: monocapa de células mesoteliales que tienen características de células epiteliales (morfología cúbica o algo aplanadas, expresión de marcadores epiteliales como citoqueratina) y secretan distintas sustancias implicadas en la regulación de la permeabilidad peritoneal y de la defensa local.

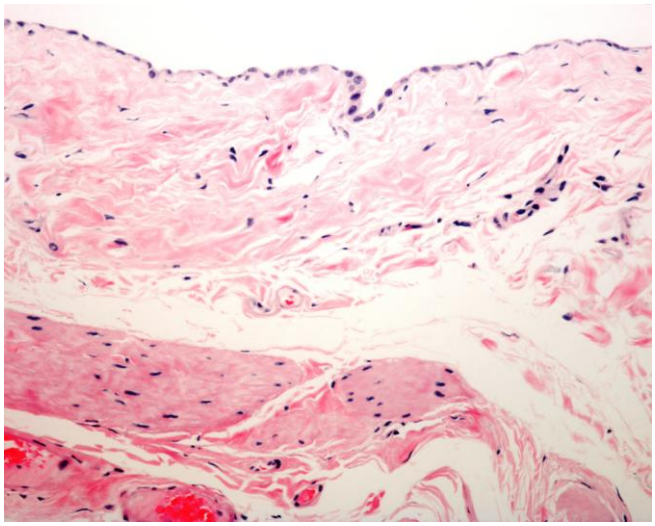
B. Membrana basal: se sitúa entre la monocapa mesotelial superficial y el submesotelio.

C. Región submesotelial: Capa de tejido conectivo que constituye la mayor parte de la membrana peritoneal. Está compuesta fundamentalmente por matriz extracelular (colágeno, fibras elásticas) de aspecto laxo y reticular, unas pocas células (fibroblastos, mastocitos y algún macrófago) y vasos sanguíneos y linfáticos. El grosor submesotelial normal es variable, alrededor de 50 µm de tamaño medio, con un límite máximo admitido de 150 µm.

D. Sistema vascular: compuesto por los vasos sanguíneos y linfáticos.

En el peritoneo visceral, el submesotelio se continúa con las estructuras viscerales y en el peritoneo parietal con tejido adiposo y músculo esquelético.

Figura 1. Membrana peritoneal de un sujeto sano. Se observa una monocapa mesotelial en la superficie y una zona submesotelial de escaso espesor y aspecto laxo.



2.2.2.- En pacientes tratados con diálisis peritoneal

La exposición del peritoneo a las soluciones convencionales de DP provoca una serie de alteraciones en su anatomía. Los principales cambios morfológicos de la membrana peritoneal descritos durante el tratamiento con estas soluciones son (Figura 2):

– Pérdida de la capa mesotelial: es la lesión más precoz y más frecuente.

– Reduplicación y engrosamiento de la membrana basal submesotelial y subendotelial (21).

– Fibrosis submesotelial: El submesotelio aumenta de grosor y adquiere aspecto homogéneo, con aumento de la matriz extracelular y de fibroblastos. En algunos pacientes, y en fases muy avanzadas, se produce un marcado depósito de matriz colagenizada, con disminución relativa del resto de elemento celulares, dando lugar al denominado desierto celular. Diversas moléculas han sido implicadas en su patogenia (factor de crecimiento transformante beta-TGF-β, factor de crecimiento vascular endotelial-VEGF, factor de crecimiento fibroblástico-FGF)(22,23). Se han descrito dos tipos de fibrosis peritoneal (24):

- *Fibrosis o esclerosis peritoneal simple*: la más frecuente y generalmente de poca intensidad. Progresa con el tiempo en diálisis y cesa cuando se abandona la técnica.
- *Peritonitis esclerosante*: más infrecuente pero más severa. Es una fibrosis progresiva, acompañada de inflamación, calcificación y depósito de fibrina que puede provocar en fases avanzadas englobamiento de las asas intestinales (esclerosis peritoneal encapsulante). Tiene elevada mortalidad y progresa a pesar de la interrupción de la DP.

– Vasculopatía hialinizante (VH)(Figura 3): Es debida a la reduplicación de la membrana basal subendotelial, de forma similar a lo que ocurre en la microangiopatía diabética (21) y se ha relacionado con la fibrosis submesotelial. Se asocia al tiempo en DP y al fallo de UF.

– Angiogénesis: aumento del número de vasos peritoneales que provoca un aumento de la superficie de intercambio (25). Este aumento es debido en parte a la producción de VEGF(26), citoquina inductora de proliferación del endotelio vascular y de un aumento de la permeabilidad vascular, ambos mecanismos implicados en el alto transporte peritoneal y el déficit de UF de los pacientes en DP (27,28). La célula mesotelial y la célula endotelial son la principal fuente de VEGF peritoneal (29).

Figura 2: Biopsia peritoneal de un paciente tratado con DP. Se observa pérdida de la monocapa mesotelial y una zona submesotelial de aspecto homogéneo y escasamente celular.

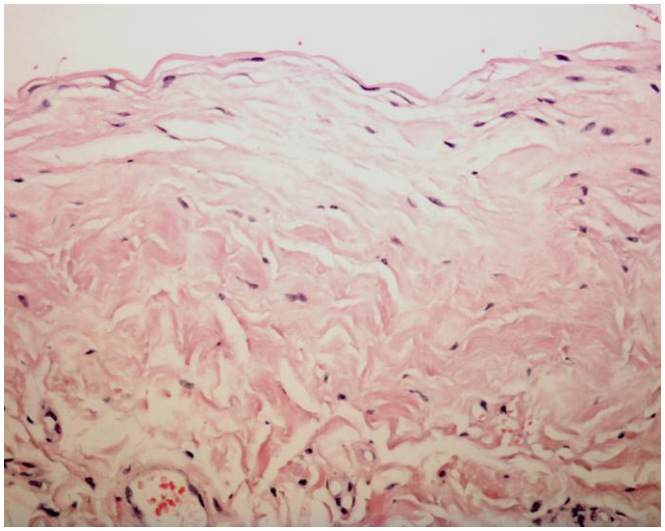
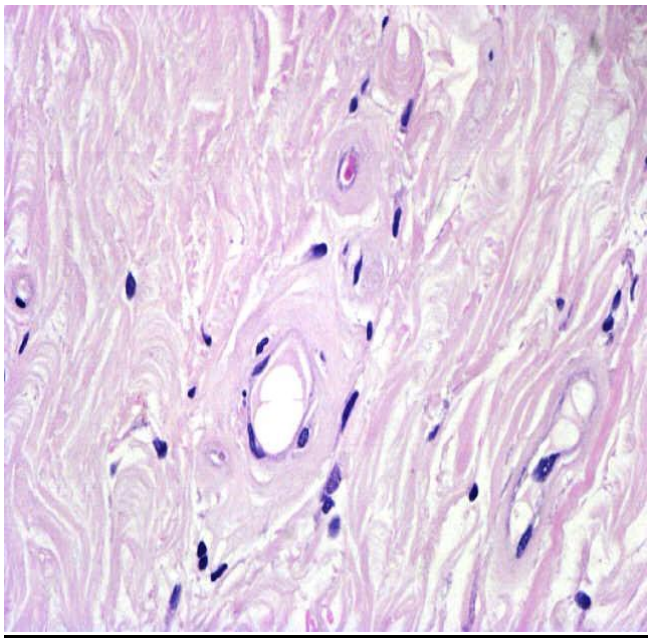


Figura 3. Vasculopatía hialinizante evolucionada en un paciente en DP



2.2.3.- En pacientes urémicos sin diálisis

Existen diversos estudios con biopsia peritoneal realizada en pacientes urémicos sin diálisis que sugieren que la uremia podría contribuir a las lesiones morfológicas que aparecen en el peritoneo de pacientes tratados con DP. Varios describen la presencia de fibrosis submesotelial y/o VH, con frecuencia de grado leve. Mateijsen y cols. (30) observaron fibrosis peritoneal en 7 de 15

pacientes antes de comenzar DP. Posteriormente, varios autores (31,32,33) han descrito que los pacientes urémicos sin DP presentan un grosor submesotelial peritoneal significativamente mayor que los sujetos control, por lo que sugieren un posible papel patogénico de la uremia en el desarrollo de fibrosis peritoneal.

Por lo que respecta a la VH, Williams y cols. (32) no la observan en sujetos controles, pero sí en un 28% de pacientes urémicos sin DP, con lesiones generalmente de grado leve-moderado. Una prevalencia similar (20.9%) es referida por Honda y cols. (33) en sujetos urémicos antes del comienzo de DP. La escasa severidad de la vasculopatía y su baja prevalencia en pacientes urémicos sugiere que probablemente los factores relacionados con la DP, pero no la uremia, sean los que juegan el papel etiopatogénico fundamental en pacientes tratados con esta técnica.

Además de la uremia, otro factor que se ha relacionado con la histopatología peritoneal en sujetos urémicos es la presencia o no de diabetes. Williams y cols. (32) observaron mayor prevalencia de VH en urémicos sin DP diabéticos que en no diabéticos, aunque fue similar en los pacientes en DP, independientemente de la presencia o no de diabetes. Más tarde, Honda y cols. (33) muestran en pacientes urémicos sin DP grados más severos de VH en diabéticos que en no diabéticos, lo que sugiere que la diabetes podría tener un papel patogénico en la VH del paciente urémico. Sin embargo, cuando estos mismos autores analizan las biopsias de pacientes en DP, no encuentran diferencias en la severidad de la VH en pacientes diabéticos o no. Esto indica que la diabetes, una vez iniciada la DP, tiene menos impacto en el deterioro de

la membrana peritoneal, siendo a partir de entonces otros factores, como las peritonitis, más determinantes.

2.3.- Transporte peritoneal

2.3.1.- Conceptos

Transporte de solutos

En los pacientes en DP, tras la introducción de la solución de diálisis en la cavidad abdominal se produce un paso de solutos del plasma al peritoneo y viceversa a través de un mecanismo de **difusión**, que finaliza cuando se equilibra su concentración en ambos compartimentos.

Transporte de agua

Parte de los solutos son eliminados junto con el agua que pasa de los capilares a la cavidad abdominal en la misma concentración que tienen en el plasma, mediante un mecanismo de **convección** (ultrafiltración). En la membrana peritoneal, el transporte convectivo se realiza a través de los poros pequeños y grandes de la pared capilar, los cuales permiten el paso de solutos de bajo peso molecular pero tienen restringido el paso de moléculas de alto peso molecular (34). Los poros ultrapequeños o acuaporinas no permiten el transporte convectivo, porque solo permiten el paso de agua, pero no el de solutos (35).

Como ya hemos comentado anteriormente, el agente osmótico más utilizado para conseguir ultrafiltración es la glucosa, existiendo como alternativas la icodextrina y los aminoácidos.

2.3.2.- Evaluación de la función peritoneal

La evaluación periódica del transporte de solutos de bajo peso molecular y de agua es fundamental.

Evaluación del transporte de solutos

Los métodos más utilizados para medir el transporte de pequeños solutos son el test de equilibrio peritoneal (PET)(36) y el cálculo del coeficiente de transferencia de masas (MTC) por el método simplificado de Garred (37), pero ambos tienen el inconveniente de que no diferencian el transporte difusivo del convectivo. El mejor método para evaluar únicamente el transporte difusivo de pequeños solutos a nivel peritoneal es el cálculo del MTC mediante el modelo matemático complejo (38,39).

La relación que existe entre la concentración de creatinina en el dializado y el plasma, denominado cociente D/P-Cr, es el valor más empleado para medir transporte de solutos, pero no diferencia el transporte difusivo del convectivo. Según la rapidez con la que se equilibra la concentración de solutos entre el plasma y el dializado de la cavidad abdominal, los pacientes se clasifican en transportadores altos o rápidos, medio-altos, medio-bajos y bajos o lentos. Los pacientes con transporte alto pierden rápidamente el gradiente osmótico por rápida absorción de la glucosa del dializado, lo que provoca que tengan menos UF, pero generan un equilibrio rápido y completo de pequeños solutos. Por el contrario, los transportadores lentos mantienen durante más tiempo el gradiente osmótico, produciendo más UF, pero equilibran de forma más lenta e incompleta la concentración de pequeños solutos. Los transportadores medios tienen características intermedias a los dos anteriores.

Evaluación del transporte de agua

El transporte de agua se calcula mediante la medición de la UF obtenida tras la realización del PET. Inicialmente, se obtenía tras realizar un intercambio de 4 horas de duración con una solución con glucosa al 2.27%/2.5%, pero más tarde, debido a la escasa capacidad osmótica de ésta se comenzó a realizar con soluciones hipertónicas de glucosa (3.86%/4.25%), según las recomendaciones de la Sociedad Internacional de DP (40). El uso de estas últimas soluciones permite además conocer el transporte de agua libre, que se mide mediante el cribado de sodio (descenso de la concentración del sodio en el dializado tras dos horas de permanencia).

2.4.- La diálisis peritoneal a corto plazo con soluciones convencionales

Durante los últimos años, se han publicado numerosos estudios de biología celular e histopatología de la membrana peritoneal que han tratado de analizar las lesiones morfológicas que pueden ser responsables de la alteración funcional peritoneal. Nuestra idea es que los estudios histológicos nos pueden permitir confirmar *in vivo* los mecanismos fisiopatológicos postulados *in vitro* o *ex vivo,* evitando con ello la extrapolación a veces incorrecta de los modelos animales.

En la mayoría de los estudios de biopsia peritoneal realizados hasta el momento en humanos (30,31,32, 41), se han encontrado con frecuencia lesiones morfológicas avanzadas, debido a que la mayor parte de ellos han sido realizados en pacientes con largas estancias en DP con problemas funcionales asociados, sobre todo fallo de UF. En este sentido, en la presente tesis se intenta conocer cuál es la correlación anatómica de los cambios funcionales presentes en los pacientes sin complicaciones en la técnica.

2.4.1- Estudios funcionales

Al inicio de DP, el tipo de transporte peritoneal es muy variable (42), siendo poco conocidos los factores que lo determinan. Algunos estudios han encontrado relación del alto transporte (AT) peritoneal inicial con factores genéticos (polimorfismos del gen de la interleuquina-6)(43) o con aspectos clínicos (edad, sexo, comorbilidad, hipoalbuminemia, función renal residual…)(44,45,46). A diferencia de lo que se describió inicialmente (6), en la actualidad no hay evidencia de que la presencia de AT peritoneal al inicio de DP (denominado AT inherente) se asocie a mayor mortalidad en DP (47), por lo que el tipo de transporte al inicio de la DP no debe ser un factor que contraindique el uso de DP a largo plazo.

El transporte peritoneal cambia con frecuencia tras el inicio del tratamiento con DP. Los cambios de la función peritoneal durante la DP con soluciones convencionales han sido estudiados por varios grupos (48,49,50), pero los que analizan su evolución a corto plazo son escasos y controvertidos. Varios autores encuentran un aumento del transporte de pequeños solutos junto con disminución de la capacidad de UF durante el primer año, mientras otros describen un descenso del transporte de solutos en este periodo. Durante el primer año en DP, las peritonitis han sido el principal factor relacionado con los cambios de la función peritoneal, sobre todo los casos de peritonitis severas (51,52).

Es bien conocido que en los pacientes con largas estancias en DP tratados con soluciones convencionales existe una relación inversa entre el transporte de pequeños solutos y agua, de manera que los pacientes con aumento del transporte de pequeños solutos tienen generalmente asociado un

fallo de la capacidad de UF. Según un estudio realizado en 574 pacientes en DP por Davies y cols. (53), la relación entre el transporte de pequeños solutos y de agua cambia cualitativamente con el tiempo en DP. Durante los primeros doce meses de tratamiento con DP, el incremento del transporte de pequeños solutos no siempre se asocia a un descenso de la UF, y posteriormente existe una disminución desproporcionada de la capacidad de UF respecto al aumento del transporte de pequeños solutos. Esta disociación entre el transporte de solutos y agua implica que los cambios cualitativos de la membrana pueden diferir en etapas precoces y tardías de la DP, y sugiere que con el tiempo en DP se producen cambios en la relación morfo-funcional peritoneal.

Lo y cols. (54) fueron los primeros en describir que los cambios iniciales de la función peritoneal dependen del tipo de transporte al inicio de la DP. Durante los primeros 18 meses de DP observaron una tendencia a la disminución del AT peritoneal y a un aumento del bajo transporte, y de forma progresiva a un aumento posterior que es independiente del tipo de transporte inicial. Otros autores también han observado que los pacientes que mostraban aumento del transporte durante el primer año en DP son los que tenían bajo transporte inicial, y viceversa (51). En un estudio posterior, Chung y cols. (55) refieren un aumento del transporte de pequeños solutos y disminución de UF durante el primer año en DP en más del 70% de los pacientes. Estos autores relacionan el estado de AT peritoneal con un estado inflamatorio y con mayor pérdida de función renal residual.

2.4.2- Estudios morfológicos

Los estudios que correlacionan función y morfología peritoneal en pacientes en DP son escasos e incluyen un número limitado de pacientes.

Habitualmente describen lesiones anatómicas avanzadas, debido a que las muestras peritoneales han sido obtenidas mayoritariamente de pacientes con deterioro funcional severo.

Las oportunidades de obtener biopsias de peritoneo en pacientes en DP son escasas debido a la invasividad de la técnica, por lo que debemos aprovechar la realización de cirugías abdominales o de un trasplante renal para su obtención. Estas dificultades hacen que pocos grupos recojan de forma sistemática muestras peritoneales de pacientes en DP y que las series sean pequeñas.

El análisis morfológico de la membrana peritoneal procedente de pacientes con cortas estancias en DP puede permitirnos interpretar mejor la respuesta peritoneal primaria y ayudarnos a conocer los mecanismos moleculares responsables del daño peritoneal inicial, permitiéndonos con ello la intervención terapéutica en fases aún reversibles.

Mateijsen y cols. (30) describieron la presencia de fibrosis, generalmente leve, en todos los pacientes en DP que estudiaron (siete pacientes con menos de dos años en DP). Posteriormente, Williams y cols. (32) mostraron un 29% de pacientes con vasculopatía hialinizante, en su mayoría de grado leve, en 58 pacientes con menos de dos años en DP.

2.5.- La diálisis peritoneal a medio-largo plazo con soluciones convencionales

2.5.1- Estudios funcionales

La evolución a medio-largo plazo del transporte peritoneal durante el tratamiento en DP con soluciones no biocompatibles ha sido estudiada por varios autores. Casi todos los estudios longitudinales describen estabilidad de

la función peritoneal en la mayoría de pacientes, observando en un 20% un aumento progresivo del transporte de pequeños solutos (AT peritoneal adquirido) y una disminución de la capacidad de UF a partir del 3-4º año. El AT adquirido se ha relacionado con menor supervivencia de la técnica y del paciente, además de preceder en ocasiones al desarrollo de peritonitis esclerosante. Davies y cols.(51) refieren un aumento del transporte de pequeños solutos y descenso de la UF ya a partir del sexto mes en DP, siendo significativo a partir del 4º año. En este estudio se demuestra que las peritonitis repetidas o graves son un factor importante asociado con estos cambios, similar a lo encontrado en otras series (56). Más tarde, este mismo grupo (53) en un amplio número de pacientes con largo seguimiento en la técnica, observan en un 25% de pacientes un rápido incremento del transporte de solutos junto con disminución desproporcionada de la capacidad de UF a partir del 4º año en DP.

Los principales factores de riesgo que se han relacionado con el desarrollo de AT y fallo de la capacidad de UF a largo plazo son una mayor exposición a soluciones ricas en glucosa y derivados en los primeros años en DP (57) y mayor comorbilidad al inicio de la técnica (44).

2.5.2- Estudios morfológicos

Como ya hemos comentado, la mayoría de grupos han analizado muestras peritoneales de pacientes en DP que han sido obtenidas de pacientes con largas estancias en la técnica o de pacientes con alteraciones funcionales severas. Las alteraciones más frecuentemente encontradas en pacientes a largo plazo durante el tratamiento con DP son la fibrosis submesotelial y la vasculopatía hialinizante (VH). Estas lesiones se han asociado además a la

aparición de fenómenos de angiogénesis peritoneal (30,31,32). Varios grupos (30,32,33) han descrito que tanto la prevalencia como la severidad de la fibrosis y la VH aumentan con el tiempo en DP, especialmente en pacientes con fallo de la membrana o largas estancias en la técnica. La presencia de fibrosis a nivel peritoneal se ha relacionado con alteraciones funcionales peritoneales (31,33) y con la peritonitis esclerosante (30).

El mecanismo fisiopatológico del AT peritoneal de pequeños solutos no es bien conocido. Inicialmente, el aumento del número de vasos a nivel de la membrana peritoneal se postuló como la alteración anatómica responsable del AT adquirido con el tiempo en DP (31). Mateijsen y cols. (30) describieron hace años en un pequeño grupo de pacientes un incremento del número de vasos peritoneales con el tiempo en DP. Pero estudios posteriores (32,58,59) han demostrado que la angiogénesis peritoneal no es un hecho constante en todos los pacientes con largas estancias en la técnica. Williams y cols (32), en la mayor serie de biopsias peritoneales publicada (130 pacientes en DP), observaron mayor densidad vascular sólo en aquellos pacientes que presentaban problemas relacionados con la técnica. Sin embargo, no encontraron diferencias significativas en el número de vasos peritoneales a distintos tiempos en DP. Otros grupos (59) han confirmado posteriormente que en pacientes en DP sin complicaciones la densidad vascular peritoneal es independiente del tiempo en diálisis.

En definitiva, todos los estudios sugieren que durante los primeros años de tratamiento, los pacientes en DP sin complicaciones no muestran alteraciones morfológicas significativas en la membrana peritoneal. Durante el tratamiento con DP, existe con el tiempo un progresivo aumento del grosor

submesotelial y de la VH, asociado a angiogénesis en los pacientes que presentan un fallo funcional de la membrana peritoneal.

2.6.- La diálisis peritoneal con soluciones biocompatibles

Múltiples estudios *in vitro* (60,61,62), *ex vivo* (63) e *in vivo* (16) han mostrado los efectos nocivos de los distintos componentes de las soluciones convencionales (glucosa y PDGs, lactato, bajo pH) sobre la membrana peritoneal, pero el impacto de las nuevas soluciones más biocompatibles sobre la anatomía y función peritoneal no está aún aclarado. Si bien algunos estudios *in vitro* y *ex vivo* muestran una mejor preservación de la morfología (64,65,66,67,68,69,70,71) y de los mecanismos de defensa peritoneales (72), así como menor inducción de transición epitelio-mesenquimal de la célula mesotelial (73), los efectos clínicos beneficiosos de las nuevas soluciones observados por algunos grupos (71,74,75,76), no han sido demostrados en pacientes en DP a largo plazo.

2.6.1.- Estudios funcionales

La evolución del transporte peritoneal con soluciones biocompatibles es objeto de controversia. Son pocos los estudios que comparan la evolución de la función peritoneal con las soluciones convencionales y con las nuevas soluciones. El uso de soluciones bajas en PDGs (71) o con bicarbonato (77) se ha asociado con un aumento del transporte de pequeños solutos y una disminución de la capacidad de UF, aunque estos hallazgos no son constantes en todos los estudios (78). Un reciente meta-análisis de varios ensayos clínicos controlados, no encuentra diferencias en el transporte de solutos ni en la capacidad de UF entre ambos tipos de soluciones.

2.6.2- Estudios morfológicos

En modelos animales se han demostrado efectos beneficiosos de las nuevas soluciones sobre la morfología peritoneal (79). Sin embargo, en pacientes en DP casi todos los estudios con biopsia peritoneal han sido realizados en pacientes tratados con soluciones convencionales (5,58), siendo muy pocos los que incluyen pacientes en tratamiento con soluciones biocompatibles (80, 81, 82). Ayuzawa y cols. (81) observaron en 11 pacientes con más de 3 años en DP lesiones leves de fibrosis y VH. Las principales limitaciones de este estudio son que incluye pacientes que recibían un tratamiento mixto con DP y hemodiálisis, y que las muestras peritoneales habían sido obtenidas por problemas relacionados con la técnica. En otra corta serie de 12 pacientes en DP tratados con soluciones biocompatibles una media de 51.9 meses, Kawanishi y cols. (82) refieren menor grado de fibrosis submesotelial y VH, con menor acúmulo de AGEs, en los pacientes tratados con soluciones más biocompatibles, cuando se comparan con pacientes urémicos sin diálisis y con pacientes tratados con soluciones convencionales.

3.- HIPÓTESIS

La variación que experimenta el transporte de solutos y agua a través de la membrana peritoneal en pacientes tratados con DP, desde el comienzo de la terapia hasta el final de la misma, sólo podrá ser explicada cuando conozcamos los cambios morfológicos que acontecen en este periodo. No siempre es necesario que exista una equivalencia entre los cambios anatómicos y funcionales, de modo que cuando el tejido peritoneal interactúa con distintos tipos de soluciones de diálisis y durante distintos periodos de tiempo, la medición de la función peritoneal puede no reflejar la realidad anatómica.

4.- OBJETIVOS

El principal objetivo de esta tesis es conocer cuál es la relación existente entre las alteraciones de la función peritoneal que se presentan en la práctica clínica diaria y las lesiones morfológicas observadas en la membrana peritoneal de los pacientes tratados con DP. Nuestra intención es investigar qué mecanismos fisiopatológicos intervienen en el fallo de la membrana peritoneal cuando ésta interactúa con soluciones de diálisis de diferente biocompatibilidad y durante periodos de tiempo variables.

Los objetivos concretos son conocer:

1.- Cómo es la función y la morfología del peritoneo cuando el paciente urémico inicia tratamiento con diálisis peritoneal. Cuál es el papel desempeñado por la uremia y otros factores en estos cambios.

2.- Los cambios que experimenta a corto plazo la función peritoneal durante la DP con soluciones de diálisis convencionales, y cuál es la lesión histológica que inicia los mecanismos patogénicos posteriores.

3.- Cuál es la relación entre las alteraciones anatómicas encontradas en pacientes tratados con soluciones convencionales a medio-largo plazo y los cambios de la función peritoneal.

4.- Qué efectos provoca sobre la función y la histopatología peritoneal en los pacientes el uso de las soluciones más biocompatibles.

5.- PACIENTES Y MÉTODOS

En los diferentes capítulos del apartado de resultados se describe detalladamente los pacientes que han sido incluidos en los distintos estudios y la metodología empleada. En el presente apartado explicaremos los aspectos comunes a todos ellos.

5.1.- Estudio de la función peritoneal

En nuestra unidad se realiza de forma periódica el test de equilibrio peritoneal (PET), realizándose mediante un intercambio con una solución que permanece 4 horas en la cavidad peritoneal. Se extrae una muestra de sangre y se toman varias muestras de efluente peritoneal (al inicio y a los 30, 60, 120, 180 y 240 minutos). La prueba se realiza en ayunas, estando el paciente clínicamente asintomático y en ausencia de hemoperitoneo o peritonitis en el último mes. La solución empleada siempre fue la misma que el paciente utilizaba en su terapia habitual.

Transporte difusivo: Modelo matemático para el cálculo del coeficiente de transferencia de masas

Para el estudio del transporte difusivo peritoneal se utiliza desde 1981 la medición del coeficiente de transferencia de masas con el modelo matemático complejo, que se detalla a continuación:

La cinética de los solutos se describe mediante un modelo que incluye un pool con un volumen constante para el paciente y un pool con un volumen variable en el dializado, como sigue:

Transferencia de masa = $d(VD\ CD)/dt = MTC\ (CB-CD) + Tr\ Qu\ CS$ (1)

siendo V = volumen de dializado o volumen de distribución para la sangre; C = concentración; D = dializado; B = sangre; t = tiempo; MTC = coeficiente de

transferencia de masa; Tr = exp (-0,0609 x $PM^{1/3}$) = coeficiente de permeabilidad (1 para urea y creatinina) y Qu= tasa de ultrafiltración (dVD/dt). El balance de masa total es:

$$VD\ CD + VB\ CB = Gt - KrCBt + CB_0VB + CD_0VD_0 \qquad (2)$$

siendo G = tasa de generación; Kr = función renal residual; 0 = valores iniciales y VB = 4/7 del peso corporal.

La resolución de la ecuación 2 para VB suponiendo una variación constante y pequeña en el CB con sustitución en la ecuación 1 da:

$$dCD/dt = 1/VD[\alpha1\alpha2 + \alpha1Gt - [MTC + Qu + \alpha1VD)CD] \qquad (3)$$

$$\alpha1 = MTC + QuTr/VB + Krt \qquad (4)$$

$$\alpha2 = CB_0Vb + CD_0VD_0 \qquad (5)$$

Como VD y Qu están en función del tiempo, un método de solución es la aproximación del diferencial mediante técnicas numéricas de diferencia finita. Esto conduce a:

$$CD_{(\alpha + 1)} = lt/VDn\ [\alpha1\alpha2 + \alpha1GTn - (MTC + Qu + \alpha1VDn)CDn] + CDn \qquad (6)$$

siendo $CD_1 = CD_0$ (condición inicial) y lt = incremento del tiempo.

La ecuación 6 está ajustada a un perfil de concentración en el sentido de mínimos cuadrados, donde el error lo da la S:

$$S = \sum_{i=1} (CDi^1 - CDi)^2$$ (valor i° calculado – valor del dato i°).

El mejor ajuste para los datos se obtiene para

$$dS/dX = 0$$

donde S es MTC en este caso.

La integración de las ecuaciones diferenciales y la determinación del MTC se realizaron mediante el método de la minimización del error cuadrático del cuarto orden de Runge y Kutta, con adaptación del intervalo de integración (subrutina DEF de la biblioteca PL-MAT de IBM). La minimización se realizó mediante el método de Powell (subrutina FMND de la misma biblioteca). El intervalo de integración fue de 1 minuto. El número de iteraciones para alcanzar el mínimo fue de cuatro.

Transporte convectivo: Cálculo de la ultrafiltración

Durante los primeros años, la capacidad de UF (medida en mililitros) se obtenía midiendo el balance negativo neto de líquido tras la realización de una pauta rígida de diálisis, consistente en cuatro intercambios diarios de dos litros, tres con una solución glucosada al 1.5% y uno con una solución glucosada al 4.25%. Con estos datos, se obtenían los valores mensuales a partir del promedio de la UF diaria.

Desde el año 2000, el transporte convectivo se mide de forma estandarizada mediante el cálculo de la capacidad de UF, según las recomendaciones de la Sociedad Internacional de Diálisis Peritoneal (83). Se calcula tras la infusión de dos litros de una solución con glucosa hipertónica (3.86-4.25%) durante 4 horas, midiendo la diferencia entre el volumen drenado y el volumen infundido (tras el peso de las bolsas de diálisis) en mililitros.

Para valorar el cribado de sodio se midió a los 60 minutos el porcentaje de descenso de la concentración de sodio con respecto a la inicial, utilizando la siguiente fórmula:

DifNa60 min = [sodio en dializado inicial – sodio en dializado a los 60 min]x100 /[sodio en dializado basal]

5.2.- Estudio del tejido peritoneal

Desde el año 2000, se realiza en nuestro hospital la recogida sistemática de muestras de peritoneo parietal de pacientes tratados con DP. Igualmente, se han recogido varias muestras de pacientes urémicos sin diálisis (en estadio ERCA-5) y pacientes en tratamiento con hemodiálisis. Las muestras de tejido peritoneal que se utilizaron como controles normales fueron obtenidas de pacientes no urémicos sin patología abdominal (donantes de riñón, casos de autopsia).

Las muestras de peritoneo parietal se tomaron de la pared anterior del abdomen y su tamaño osciló entre 10 y 25 mm cada lado. Se fijaron inmediatamente en formol para evitar su desecación y se estiraron suavemente sobre una superficie, preferiblemente de corcho, para evitar su retracción Tras la fijación durante 12–24 horas se procesaron las muestras, para lo cual se cortan en finas secciones de similar tamaño y posteriormente se incluyen en parafina. Finalmente son teñidas con hematoxilina-eosina y tricrómico de Masson. Para los estudios inmunohistoquímicos se empleó un método indirecto con sistema de visualización mediante polímero. Previamente, y con objeto de desenmascaramiento antigénico, las secciones fueron calentadas en una solución de acido cítrico (pH 6) utilizando un microondas

6.- RESULTADOS

Relacionados con el tratamiento con soluciones de diálisis convencionales:

Capítulo 1. El transporte de pequeños solutos y agua al inicio de la diálisis peritoneal

6.1.1.1- “La ultrafiltración y el transporte de pequeños solutos al inicio de la diálisis peritoneal. Cuestionando el paradigma de la función peritoneal”

Selgas R, Bajo MA, Cirugeda A, del Peso G, Valdés J, Castro MJ, Sánchez S, Fernández–Reyes MJ, Hevia C, Gil F, Aguilera A, Ortiz J, Alegre L, Álvarez V, Sánchez–Tomero JA.
Peritoneal Dialysis International 2005; 25: 68-76

Este trabajo responde al objetivo 1

La ultrafiltración y el transporte de pequeños solutos al inicio de la diálisis peritoneal. Cuestionando el paradigma de la función peritoneal.

La función peritoneal al inicio de la diálisis peritoneal no es bien conocida.

Objetivo: Establecer el comportamiento de la función peritoneal al comienzo de la DP.

Métodos: Se analizan los coeficientes de transferencia de masas (MTC) de urea y creatinina, así como la UF estandarizada al inicio del tratamiento con DP (primeras dos-seis semanas) en 367 pacientes.

Resultados: Los valores medios fueron: MTC-urea 22.9±7.04 ml/min, MTC-Cr 10.31±4.68 ml/min y UF 896±344 ml/4h. Estos parámetros no se asociaron con la talla, superficie corporal, sexo, diabetes ni enfermedad renal de base. El MTC-Cr y la UF se asociaron de forma significativa, aunque con escasa correlación ($r=-0.3$, $p=0.001$). La edad se correlacionó inversamente con la capacidad de UF ($r=-0.15$, $p=0.003$) y con el MTC-urea ($r=-0.11$, $p<0.05$). En el análisis de regresión logística, una UF<400 ml/4h se asoció de forma independiente con un elevado MTC-creatinina y con mayor edad. En los pacientes con menor UF se observó menor prevalencia de diabetes. Cuando se estudiaron los quintiles de MTC-creatinina, no se encontró el patrón inverso esperado de la capacidad de UF. La UF fue similar entre el segundo y el tercer-cuarto quintil, mientras que el MTC-creatinina varió de 6.71 a 13.54 ml/min.

Conclusiones: Al inicio de DP, las características de la función peritoneal son muy variables, existiendo una débil relación entre el transporte de solutos y agua. La edad se asocia con baja UF, y esta relación es independiente del transporte de solutos.

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ULTRAFILTRATION AND SMALL SOLUTE TRANSPORT AT INITIATION OF PD: QUESTIONING THE PARADIGM OF PERITONEAL FUNCTION

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♦♦***Background:*** **Human peritoneal function on commencing peritoneal dialysis (PD) is not yet adequately understood. The objective of this study was to determine peritoneal functional patterns on commencing PD.**

♦♦***Methods:*** **367 end-stage renal disease (ESRD) patients on PD for the first time were studied between their initial second to sixth weeks on PD. Urea and creatinine mass transfer area coefficients (MTAC) and standardized ultrafiltration (UF) capacity were determined.**

♦♦***Results:*** **Mean parametric values were MTAC urea 22.9 ± 7.04 mL/min, MTAC creatinine 10.31 ± 4.68 mL/min, and UF 896 ± 344 mL. Gender, patient size, and diabetes or kidney disease did not affect these parameters. The relationship between values of MTAC creatinine and UF reached statistical significance, although with a low value for Pearson's coefficient ($r = -0.30$, $p = 0.001$). Age showed a significant inverse linear correlation with UF capacity ($r = -0.15$, $p = 0.003$) and MTAC urea ($r = -0.11$, $p < 0.05$). Logistic regression analysis demonstrated that UF below 400 mL was independently related to a high MTAC creatinine and older age. Diabetes was least frequent in patients with the lowest UF. However, in the analysis of MTAC creatinine quintiles, UF values did not follow the expected inverse pattern. The lack of differences in UF between the second and third to fourth MTAC creatinine quintiles is remarkable; MTAC creatinine ranged from 6.71 to 13.54.**

♦♦***Conclusions:*** **The functional characteristics of human peritoneum varied markedly and there was a less intense than expected relationship between solute and water transports. This mild inverse relationship is intriguing and suggestive of the necessity of redefining some basic concepts. Age was associated with a lower peritoneal UF capacity, in part independently of small solute transport.**

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KEY WORDS: Human peritoneum; peritoneal function; ultrafiltration; diffusion transport; baseline peritoneal conditions.

Peritoneal dialysis (PD) has become an alternative to hemodialysis for treatment of end-stage renal disease (ESRD). However, several aspects remain to be adequately defined, particularly those that may determine the continuity of the treatment. One of these is the functional condition of the peritoneum when PD is initiated (1–5). Very recently, this issue received particular attention in a paper based on the Australia and New Zealand Dialysis and Transplant Association Registry (6). Some authors have suggested that systemic inflammation may influence peritoneal function at these stages, although no conclusive results have yet been reached (7).

One of the paradigms of long-term peritoneal function is the inverse relationship between small solute and water transports (1,4,8). However, clinical observations at the earliest stages of PD have reported isolated but remarkable exceptions that require explanation. These cases are characterized by a simultaneous increase in both transports or, on the contrary, a decrease in the two transports.

Importantly, patients with high solute transport characteristics have been reported to have a poorer prognosis with PD, although their confounding comorbid conditions might have affected the interpretation of the data (8–10). If peritoneal function may decide the patient's future, its reasons and determinants should be known.

The objective of the present study was to review peritoneal functional patterns on commencing PD in a large series of patients to establish "normality" and the intrinsic relationships between the different parameters.

PATIENTS AND METHODS

The series was comprised of 436 ESRD patients (age 53.3 ± 16.5, 219 men) initiating PD treatment for the first time between 1980 and 2001. Selection was based on their performance in a peritoneal functional study during the second to sixth weeks of employing the peritoneum for dialysis; exclusion criteria were no abdominal infections, major surgery, or hemorrhagic injuries in their entire life. Before beginning PD, 61 ofthe 436 patients had undergone hemodialysis treatment and 8 patients had been transplanted and shown chronic allograft disease. Although no statistical differences were found between these three groups (data not shown), the 69 hemodialysis and transplanted patients were definitely excluded from the analysis in order to exclude any influence caused by long-term uremia.

After excluding these patients, the remaining selected 367 patients constituted the current series. The cause of ESRD in these patients was glomerulonephritis in 53, tubulointerstitial nephropathy in 69, polycystic kidney disease (PCKD) in 38, type 1 diabetes in 38 and type 2 diabetes in 59 (diabetes was not considered the cause of ESRD in 6 others), nephrosclerosis in 39, ischemic nephropathy in 11, systemic disease (systemic lupus erythematosus, scleroderma, vasculitis, and amyloidosis) in 32, hereditary in 4, of unknown origin in 21, and other causes in 3.

The peritoneal kinetic study consisted of a 4-hour dwell-time peritoneal exchange, taking six peritoneal effluent samples (at 0, 30, 60, 120, 180, and 240 minutes) and one blood sample. During the first years we took two separate blood samples and averaged the results. That practice was eliminated after demonstrating identity between the two samples, insofar as determining urea and creatinine. During each functional study, patients were fasting and received no drugs except low doses of subcutaneous insulin, if necessary. In all patients, the urea and creatinine dialysate-to-plasma ratios [D/P; classic peritoneal equilibration test (PET)] were accessible. Beginning in 1981 (when the PET was still unknown), we calculated the peritoneal mass transfer area coefficient (MTAC) value in order to express solute transport. A previously described mathematical model (1) was applied to calculate the peritoneal MTAC, in milliliters per minute, of the solutes. This coefficient represents the isolated diffusive capacity of the membrane under theoretically infinite dialysate flow. In summary, solute kinetics is described by a single-pool constant volume model for the patient and by a variable volume dialysate pool as follows:

$$d(VD\ CD) / dt = MTAC\ (CB - CD) + Tr\ Qu\ CB,$$

where VD is the volume of dialysate, CD the concentration in dialysate, t is time, CB the concentration in blood, Tr the transmittance coefficient (1 for urea and creatinine), and Qu is dVD/dt [ultrafiltration (UF)]. The total mass balance yields

$$VD\ CD + VB\ CB = Gt - Kr\ CB\ t + CB_0\ VB + CD_0\ VD_0.$$

In the second equation, G is the generation rate (total solute content in 24-hour urine and dialysate with the patient in steady state), Kr the residual renal function, 0 the initial values, and VB the distribution volume (4/7 of body weight). In this equation, which defines the mass balance (steady state of the solute), the inclusion of G and VB takes accounts of the weight of the patient and integrates that value in the MTAC data.

The resolution of the model is carried out by the integration of differential equations and the minimization of the fourth-order quadratic error method of Runge and Kutta. This model was validated for peritoneal kinetics and MTAC calculation in 1977 (1).

During the 1980s, we performed peritoneal kinetic studies during the second week on PD. After too early kinetic studies were questioned, in 1990 we moved the baseline kinetic study to the fourth to sixth weeks. At that time, we also changed the glucose content of the dialysate for the kinetic study from 1.36% to 2.27%. We (data not shown) and others (5) have demonstrated that kinetic studies performed using dialysates with different glucose contents produce similar values for MTACs (values of $r > 0.95$ in regression analysis).

PERITONEAL UF CAPACITY MEASUREMENT

This value represents mostly the convective transport capacity and is expressed by the net negative balance (weighing the bag after drainage) using a 2-L 3.86% glucose exchange for 4 hours in the peritoneum. Our patients were asked to record the net balance of each exchange, and these were averaged at the sixth week on PD. For patients who rarely used 3.86% glucose exchanges, and especially for those who did not use these exchanges at all (although we asked them to use the high glucose dialysate sporadically for the purpose of measurement), the quantification has unavoidably been made with fewer values. Only patients with appropriate catheter drainage function (draining more than 2 L in 20 minutes or less) were considered. This methodology for UF quantification is totally independent of the MTAC procedure.

Theoretically, the patient's height might determine the proportion between size and surface in the

abdominal cavity. In the sphere that represents the abdominal cavity, the surface/volume ratio diminishes with the increase in volume (*i.e.*, 1.24 m^2/0.42 m^3 = 2.95, vs 20 m^2/26.7 m^3 = 0.740). In agreement with this, smaller individuals may have a relatively larger peritoneal surface area. In consequence of this, contact with the dialysate would be more efficient in smaller patients. Moreover, these smaller patients attain a higher hydrostatic pressure, caused by the same amount of infused dialysate (2 L) filling a relatively smaller peritoneal cavity. These two physical conditions, larger surface and more pressure from the cavity toward the capillaries, might diminish UF capacity and increase glucose absorption in the peritoneum of smaller individuals. For these reasons, we analyzed the influence of the patient's height and body surface area on UF capacity. The mean height of our population was 1.62 ± 0.09 m (median 1.62 m, range 1.32 – 1.87 m).

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS 10.0 and 11.5 versions (SPSS, Chicago, Illinois, USA).

The one-sample Kolmogorov–Smirnov test procedure was used to compare the observed cumulative function for the different variables analyzed (MTACs and UF) against a specified theoretically normal distribution. For mean or median comparisons, parametric and nonparametric tests were applied according to the type of distribution of the variable. Linear regression analysis (calculating both Pearson and Spearman coefficients) was also performed among the peritoneal functional parameters.

To make comparisons, a control group was defined according to the concept that nonsystemic kidney diseases (specifically glomerulonephritis and interstitial causes, 122 patients) would not influence peritoneal functional parameters. In contrast, PCKD (38 patients), hypertensive–vascular–systemic disease (39, 11, and 32, respectively), and diabetes (97 patients) were considered to have the potential to affect peritoneal function. Among these last groups, the only significant difference in demography was the older mean age in patients with vascular causes of ESRD (nephrosclerosis and ischemia) and type 2 diabetes, relative to the control group. Values less than 0.05 were considered statistically significant.

Due to the clinical implications that peritoneal parameters may have on patient and technique survival, we decided to study the percentiles of these values in order to compare nonarbitrary, selected extreme values. An UF capacity below 400 mL is considered to mean there is an inability to provide sufficient negative water balance (11,12). For this reason, the group with UF higher than 400 mL was divided into quartiles, so the UF capacity was divided into five final categories. Small solute transport, represented by MTAC creatinine, was divided into quintiles with the same purpose: to compare extreme groups.

Univariate and stepwise multivariate analyses were performed to explore the determining factors for the transport parameters. The two extreme categories of MTAC creatinine and UF were considered the dependent variables. The independent variables are shown in appropriate tables.

RESULTS

GENERAL DATA

The MTAC urea was the only peritoneal functional parameter to show a normal distribution. Distribution of the other peritoneal parameters was not normal (Figure 1). The Kolmogorov Smirnov Z, skewness, and kurtosis coefficients are displayed in the figure.

Mean values for the transport parameters in the overall cohort of patients were, for MTAC urea 22.9 ± 7.04 mL/minute (range 5.3 – 46 mL/min), for MTAC creatinine 10.31 ± 4.68 mL/min (range 1.7 – 28.8 mL/min), and for UF 896 ± 344 mL (range 150 – 2100 mL).

Gender did not determine differences among patients for any of the three parameters (data not shown). Diabetic patients showed values similar to those of the control group for MTAC urea (23.9 ± 7.1 mL/min in diabetic patients vs 22.9 ± 7.4 mL/min in controls, NS), MTAC creatinine (respectively, 10.6 ± 4.7 vs 10.5 ± 5.2 mL/min, NS), and UF (respectively, 931 ± 350 mL vs 884 ± 364 mL, NS). Type 1 versus type 2 diabetes showed the following data, none of which reached significance: MTAC urea (25.1 ± 7.3 mL/min in type 1 patients vs 22.8 ± 6.8 mL/min in type 2 patients, NS), MTAC creatinine (respectively, 11 ± 6 vs 10.1 ± 3.6 mL/min, NS), and UF (respectively, 1004 ± 395 mL vs 907 ± 292 mL, NS).

Patients with PCKD did not show significantly different values relative to the control: MTAC urea (23.3 ± 6.8 vs 22.9 ± 7.4 mL/min, NS), MTAC creatinine (9.2 ± 4.2 vs 10.5 ± 5.2 mL/min, NS), and UF (924 ± 332 mL vs 884 ± 364 mL, NS).

The relationship between patient size (height and body surface area) and UF or MTAC parameters did not disclose significant values. Regression analysis revealed extremely low r values (r = 0.03 and 0.04 respectively, NS). The mean height value for the different categories was almost identical (Table 1). When we adjusted UF capacity for height (UF × height in meters), no new sig-

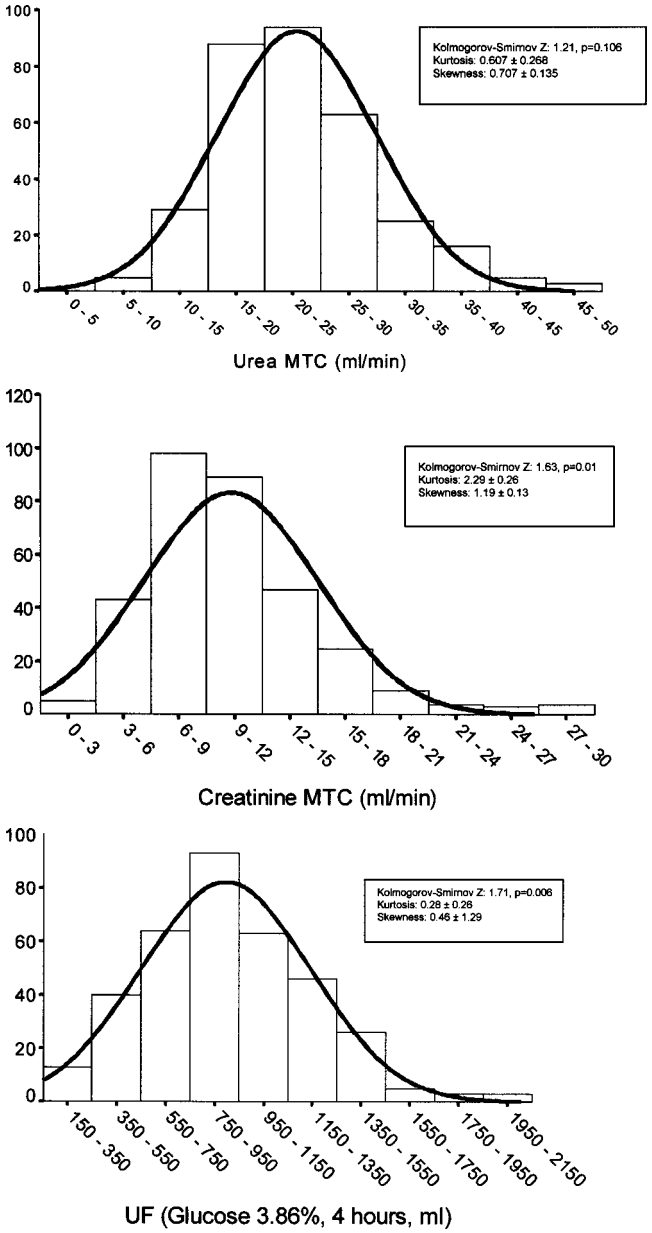

Figure 1 — Distribution of values of different peritoneal functional parameters (Gaussian curves). Mass transfer area coefficient (MTC) of urea distribution is the only curve with normal distribution. Kolmogorov–Smirnov Z, kurtosis, and skewness coefficients are displayed. UF = ultrafiltration.

nificant values were found in the regression analysis. Height and patient size were not taken into account in the final analysis. Analysis for gender also identified no significant differences.

ANALYSIS OF CATEGORIES DETERMINED BY UF VALUE

The differences in MTAC creatinine in the different UF categories reached statistical significance (ANOVA). However, it should be noted that this inverse relationship was not significant when the second and third, and third and fourth quartiles were compared to each other respectively (Table 1). These findings mean that UF values of 701 – 900 mL and 901 – 1150 mL did not reflect differences in MTAC creatinine. The low values of the Spearman and Pearson coefficients reflect these findings ($r = -0.30$, $p < 0.001$; Figure 2). Figure 2 shows the distribution of points along both axes, reflecting the poor individual correspondence between the two values.

Moreover, urea transport showed significant differences only between the second and fourth quartiles, with the other results being statistically nonsignificant.

There was a significant inverse linear correlation between age, UF capacity ($r = -0.15$, $p = 0.003$), and MTAC urea ($r = -0.11$, $p < 0.05$), with very low values for the r coefficient as well.

The urea and creatinine MTAC values maintained a significant positive correlation in each UF category, although with different r values (0.68 – 0.82). Figure 3 shows the significant linear correlation between the two parameters in the 367 patients ($r = 0.78$).

Table 2 shows the results of logistic regression analysis taking as the dependent variable the lowest UF. Multivariate analysis relative to this low UF category (29 patients) showed an independent and significant association with higher MTAC creatinine [odds ratio (OR) 1.15, confidence interval (CI) 1.06 – 1.24, $p = 0.003$] and older age (OR 1.03, CI 1.003 – 1.06, $p = 0.02$) after adjusting for diabetic status. Table 3 shows the univariate analysis using the highest UF capacity as the dependent variable. Subsequent multivariate analysis showed that belonging to this group was independently and significantly associated with lower MTAC creatinine (OR 0.85, CI 0.79 – 0.92, $p = 0.0001$) and younger age (OR 0.98, CI 0.96 – 0.99, $p = 0.04$).

ANALYSIS OF MTAC CREATININE QUINTILES

The values for MTAC urea maintained a good degree of correlation between each quintile and its superior quintile (the difference was always statistically significant). Patient age did not show differences. However, UF values did not follow the expected inverse pattern. In fact, the lowest quintile was the only one that showed significant differences relative to each of the others. The lack of differences in UF (all of the means were approximately 900 mL) between the second and third to fourth quintiles, with a range in MTAC creatinine from 6.71 to 13.54, is remarkable (Table 4). In summary, only the two extreme MTAC creatinine quintiles showed a truly significant distance insofar as UF capacity is concerned. Multivariate analysis relative to the highest MTAC creatinine

TABLE 1
Demography and Peritoneal Functional Data of Patients According to Ultrafiltration Capacity (UF) Categories (Mean±SD)

UF category (mL)	MTAC urea (mL/min)	MTAC creatinine (mL/min)	UF (mL)	Age (years)	Height (m)
<400 (*n*=29)	24.2±7.5	13.7±4.4[b]	325±81[e]	58.5±15.3	1.62±0.09
401–700	23.6±7.2	11.1±4[c]	603±87[e]	53.4±15.2	1.62±0.09
701–900	24.1±6.6[a]	10.8±4.7[d]	827±57[e]	53.6±16.6	1.63±0.1
901–1150	22.2±7.9	9.7±5.2[b]	1039±52[e]	55.4±15.1	1.61±0.08
>1150	20.8±6[a]	8.2±4[b,c,d]	1379±205[e]	49.2±17.9	1.62±0.09

MTAC = mass transfer area coefficient.
[a,b,c,d] $p < 0.05$. Statistically significant differences expressed by paired letters.
[e] Differences among the UF values among quintiles are, by definition, statistically significant.
UF in mL/3.86% glucose, 4-hour dwell time.

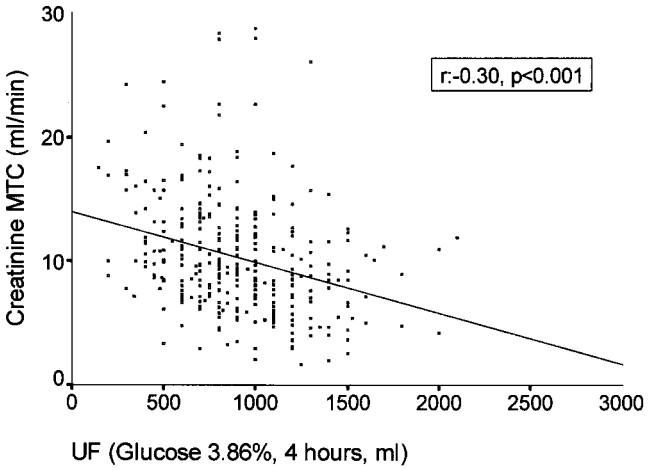

Figure 2 — Linear regression analysis between mass transfer area coefficient (MTC) of creatinine and ultrafiltration capacity (UF) for 367 patients. The correlation coefficient (*r*) was –0.30.

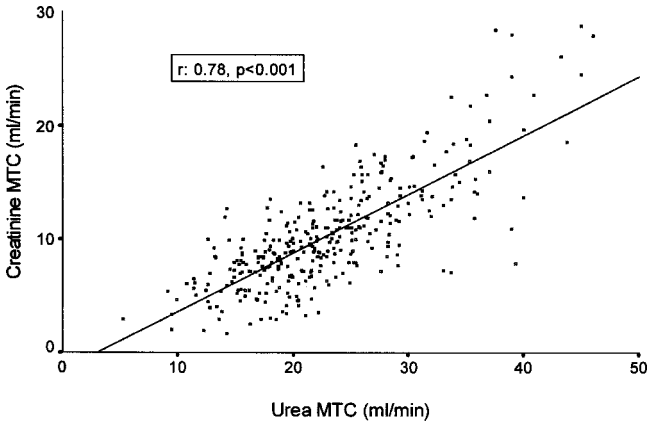

Figure 3 — Linear regression analysis between mass transfer area coefficients (MTC) of creatinine and urea for 367 patients. The correlation coefficient (*r*) was positive and reached a value of 0.78.

quintile (highest transporters) did not add additional information to the univariate analysis (Table 5). With respect to the lowest MTAC creatinine quintile (lowest transporters, Table 6), MTAC urea and UF capacity were independently associated with MTAC creatinine. Both Tables 5 and 6 show that age is not significantly related to peritoneal transport of creatinine.

DISCUSSION

The present study confirms that peritoneal function varies markedly among patients in the beginning of PD, and the existing paradigm that UF capacity is inversely related to small solute transport does not explain this variation.

Peritoneal function at baseline has been addressed by several authors (7–9) but reached a new order of magnitude after the publication of data from more than 3000 patients starting PD (6). The main findings of this study substantiate the functional diversity and the association of higher transport status with racial origin, older age, and lower body mass index. The limitation of that paper is that data were obtained along the first 6 months on PD and included patients who suffered peritonitis prior to the peritoneal study. According to the effects of peritonitis on the peritoneum, it cannot be ruled out that affected patients showed functional changes after such episodes, and the values altered the results such that they were no longer strictly baseline data.

Our data show noteworthy differing distribution of peritoneal functional parameters. Whereas MTAC urea maintained normal distribution, MTAC creatinine and UF did not. Interpreting that only effective vascular surface area is the determinant seems not sufficient to explain differences between urea and creatinine (4), and suggests other system components play a role. Concerns

TABLE 2
Univariate Logistic Regression Analysis for Ultrafiltration Deficiency (Capacity <400 mL/3.86% Glucose Exchange)

Variable	Relative risk (95% CI)	*p* Value
Age	1.02 (0.99–1.04)	0.07
Male/female	0.89 (0.41–1.91)	0.77
MTAC urea	1.02 (0.97–1.08)	0.33
MTAC creatinine	1.13 (1.05–1.2)	0.0006
MTAC creatinine highest quintile versus other quintiles	4.67 (1.99–10.9)	0.0004
Control group versus other causes of renal insufficiency	0.59 (0.27–1.27)	0.18
Diabetes		
No vs yes	0.27 (0.08–0.92)	0.03
No vs type 1	0.24 (0.03–1.86)	0.17
No vs type 2	0.29 (0.06–1.25)	0.09
Type 1 vs type 2	1.18 (0.1–13.4)	0.89

MTAC = mass transfer area coefficient.

TABLE 3
Univariate Regression Analysis for Ultrafiltration Capacity >1150 mL/3.86% Glucose Exchange (Fourth Quartile)

Variable	Relative risk (95% CI)	*p* Value
Age	0.98 (0.96–0.99)	0.01
Male/female	1.55 (0.93–2.56)	0.08
MTAC urea	0.94 (0.90–0.98)	0.004
MTAC creatinine	0.84 (0.78–0.92)	<0.0001
MTAC creatinine lowest quintile versus other quintiles	3.12 (1.74–5.59)	0.0001
MTAC creatinine lowest quintile versus highest quintile	8.3 (2.94–23.4)	0.0001
Control group versus other causes of renal insufficiency	0.83 (0.49–1.4)	0.49
Diabetes		
No vs yes	1.19 (0.69–2.04)	0.52
No vs type 1	1.51 (0.7–3.24)	0.29
No vs type 2	1.02 (0.52–1.98)	0.95
Type 1 vs type 2	0.67 (0.26–1.69)	0.4

MTAC = mass transfer area coefficient.

TABLE 4
Demography and Peritoneal Function Data of Patients According to Mass Transfer Area Coefficient (MTAC) of Creatinine Quintiles

MTAC creatinine quintile (mL/min)	MTAC urea (mL/min)	MTAC creatinine (mL/min)	Ultrafiltration (mL)	Age (years)
Low transporters (0–6.7)	16.5±4.1[a]	4.9±1.3[h]	1094±306[e]	53.2±18.1
Low-average transporters (6.71–8.6)	20.1±5.1[a,b]	7.7±0.5[h]	907±294[e,f]	56.2±16.3
High-average transporters (8.61–10.6)	21.4±3.6[a,b,c]	9.5±0.5[h]	848±348[e]	53±16.3
High transporters (10.61–13.54)	24.7±4.7[a,b,c,d]	11.9±0.8[h]	895±368[e,g]	52.7±15.3
Very high transporters (>13.54)	31.8±6[a,b,c,d]	17.5±4[h]	724±288[e,f,g]	50.4±16.7

[a,b,c,d,e,f,g] $p < 0.05$.

[h] The differences among MTAC creatinine were statistically significant by definition. Other statistically significant differences are expressed by paired letters.

TABLE 5
Univariate Regression Analysis for the Highest Mass Transfer Area Coefficient (MTAC) of Creatinine Quintile (>13.54 mL/min)

Variable	Relative risk (95% CI)	*p* Value
Age	0.98 (0.97–1.04)	0.14
Male/female	0.88 (0.51–1.52)	0.65
MTAC urea	1.39 (1.28–1.51)	<0.0001
UF capacity	0.997 (0.996–0.998)	<0.0001
Lowest UF capacity versus other UF categories	4.67 (1.99–10.9)	0.0004
Lowest UF capacity versus highest UF	13.7 (4.1–46.2)	0.0001
Control group versus other causes of renal insufficiency	0.78 (0.44–1.37)	0.39
Diabetes		
No vs yes	1.35 (0.75–2.41)	0.31
No vs type 1	1.76 (0.79–3.95)	0.16
No vs type 2	1.12 (0.55–2.3)	0.74
Type 1 vs type 2	0.63 (0.24–1.68)	0.36

UF = ultrafiltration.

TABLE 6
Univariate Regression Analysis for the Lowest Mass Transfer Area Coefficient (MTAC) of Creatinine Quintile (<6.7 mL/min)

Variable	Relative risk (95% CI)	*p* Value
Age	1.004 (0.98–1.01)	0.96
Male/female	1.34 (0.78–2.37)	0.28
MTAC urea	0.73 (0.67–0.80)	<0.0001
UF capacity	1.002 (1.001–1.003)	<0.0001
Highest UF versus other UF categories	3.12 (1.74–5.59)	0.0001
Control group versus other causes of renal insufficiency	0.78 (0.44–1.37)	0.39
Diabetes		
No vs yes	0.91 (0.49–1.6)	0.76
No vs type 1	1.14 (0.48–2.67)	0.75
No vs type 2	0.78 (0.37–1.66)	0.53
Type 1 vs type 2	0.68 (0.24–1.95)	0.48

UF = ultrafiltration.

about the influence of intraperitoneal pressure (13) should not be relevant to our data since all studies were performed with the patients in a sitting position. This assures maximum intra-abdominal pressure, close contact between dialysate and visceral peritoneum, and homogenous fluid distribution. Analysis of patient height did not reveal a significant relation to peritoneal functional parameters (14). The significant relationship between higher creatinine transport and lower body mass index referred to in another paper is not confirmed by our data and is difficult to explain (6).

Relative to UF capacity, the human peritoneum is spontaneously also variable. Twenty-nine patients (8%) had an UF capacity lower than 400 mL (in later periods known as type I UF failure), which suggests, as we hypothesized (15), that a true category of inherent peritoneal UF deficiency exists. At the other extreme, the highest UF quartile (UF > 1100 mL) represents "the highest water transporters." Glucose gradient is the main but not the unique determinant of peritoneal UF. Other patient conditions may influence this hypothesis. The diversity may also explain the relatively weak inverse (r = 0.30) relationship between UF and MTAC creatinine. Ultrafiltration categories maintained fidelity to the paradigm much more than did MTAC creatinine categories, although extreme categories of the latter parameter are significantly related to the inverse UF category. Probably both phenomena, diversity and weak relationship, are the consequence of factors existing just at the beginning of PD. The cause of ESRD, diabetic status, and

others neither determine UF/MTAC values nor influence their relationship. We found that a high UF is inversely and independently associated with age and MTAC creatinine, in both univariate and multivariate analyses.

The association between older age and higher transporters has been communicated (6). Both direct (6) and indirect (present data) evidence is very suggestive of an inverse relationship between age and peritoneal UF capacity, mediated in part by a higher solute transport capacity. The association between age and UF, with partial independence of solute transport, makes it improbable that the relationship is only conditioned by a vasoactive phenomenon. Age-conditioned membrane characteristics might influence UF capacity, but there is no information suggesting that peritoneal components are modified by age. Two papers have suggested that peritoneal permeability in the course of PD increases more in older than in younger people (16,17). Uremic status has been related to peritoneal anatomical differences, but age has not been analyzed relative to submesothelial thickness (18). Our biopsy studies have not confirmed differences between uremic patients and controls (19). Taken together, our results on peritoneal function (present) and biopsy data (19) are poorly suggestive that acutely or chronically acquired kidney disease is associated with peritoneal anatomical or functional differences.

The complexity of the relation between peritoneal transport of water and small solutes is also exemplified in patients who show both transports to be unusually elevated. The demonstration that a higher solute transport may coincide with a higher UF capacity undermines the classic paradigm. To explain this feature in the early stages of PD, we need to consider peritoneal water transport the result of bidirectional forces, including water back-filtration rate. Since the remaining structures in the area are permeable to water, this rate is determined mainly by the hyaluronan layer synthesized by mesothelial cells (20). A significant relationship between the number of vessels in submesothelial tissue and mass transport of glucose/UF capacity (21) has been reported, but not with that of creatinine. On the other hand, the expression of aquaporin-1 by peritoneal endothelial cells has also been related to water transport capacity (22), although no data have yet been published in humans. Very recently, the association of ENOS4 (a/b) gene polymorphism with basal peritoneal permeability in Chinese patients was reported (23). Genetic conditions of peritoneal capillary composition may be influencing solute and water transports.

We recognize some limitations of our study that may partially modify interpretation of the results: first, our study population is relatively small compared to the recently published data of more than 3000 patients (6); second, alternative models to calculate MTAC have been developed but would be unlikely to give significantly different values; third, UF estimations were not made at the same time the peritoneal kinetic study was performed, but rather were averaged for measurements made by the patients in their homes (We believe, however, that these UF values are valid due to the multiple measurements obtained and the standardized conditions applied.); and fourth, we have not taken into account the influence of peritoneal residual volume, lymphatic absorption, or aquaporins in calculating UF and MTAC values. However, the detailed nature of our peritoneal kinetic studies and the extension of our series using the same methodology over a 24-year period, make it likely that it is very representative of what peritoneal function is and what the relationships between the parameters used to evaluate it are, at initiation of PD.

New insights into the response of peritoneum and mesothelial cells to PD suggest that some of our current concepts will need to be redefined when new technologies, such as *in vivo* video microscopy and immunocytochemistry (24,25), can be applied and contrasted with exact information on the composition of peritoneum (18,19).

In summary, the functional characteristics of human peritoneum varied markedly and there is a less intense than expected relationship between solute and water transports. This mild inverse relationship is intriguing and suggestive of the necessity of redefining some basic concepts applicable to transport studies performed early in the course of peritoneal dialysis.

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6.1.1.2.- "Factores de riesgo responsables del fallo de ultrafiltración en etapas precoces de la diálisis peritoneal".

Selgas R, Bajo MA, Castro MJ, del Peso G, Aguilera A, Fernández-Perpén A, Cirugeda A, Sánchez-Tomero JA.

Peritoneal Dialysis International 2000; 20 (6): 631-636

Este trabajo responde a los objetivos 1, 2 y 3

Factores de riesgo responsables del fallo de ultrafiltración en etapas precoces de la diálisis peritoneal.

Objetivo: Definir los factores de riesgo del fallo de ultrafiltración durante las etapas iniciales de la diálisis peritoneal.

Métodos: Se realizó un análisis retrospectivo de la función peritoneal en un grupo de pacientes en DP del Hospital La Paz. 19 de 90 pacientes con DP a largo plazo requirieron un descanso peritoneal para recuperar la capacidad de UF: 8 lo necesitaron antes del 3º año (grupo de fallo de UF precoz o grupo de estudio), y 11 de forma tardía (grupo de fallo de UF tardío). Los 71 pacientes restantes, con función peritoneal estable, constituyeron el grupo control. Se midió una vez al mes la capacidad de UF peritoneal en condiciones estándar y el transporte peritoneal de pequeños solutos una vez al año**.**

Resultados: No se identificó ninguna condición al inicio de DP o durante el tratamiento como causa del fallo de UF. No hubo diferencias en las características entre el fallo de UF precoz y los otros dos grupos, excepto por una mayor prevalencia de diabetes en el grupo de estudio. La función renal residual descendió en todos los grupos en los primeros dos años, con una pérdida rápida durante el tercer año en el grupo de estudio, que coincidió con el fallo de UF. El transporte de solutos y agua basal fue similar en los tres grupos. Tras el 2º año en DP, los pacientes del grupo de estudio mostraron significativa menor UF y mayor coeficiente de transferencia de creatinina que los del grupo de fallo de UF tardío. Los pacientes diabéticos del grupo control mostraron una UF estable durante todo el tiempo. Durante el 2º año en DP, el grupo de estudio precisó un aumento de la concentración de glucosa en el dializado de

un 3.4 ± 0.5% en el grupo de estudio y de un 25.5 ± 24.2% en el grupo de fallo de UF tardío. Los días acumulados de peritonitis aguda (días de líquido turbio) fueron similares en los tres grupos al año y a los 2 y 3 años en DP. Los diabéticos del grupo control tenían significativamente menos días acumulados de peritonitis que el grupo de estudio y que los no diabéticos del grupo control ($p = 0.016$).

Conclusiones: Los resultados sugieren que algunos pacientes en DP desarrollan fallo de UF tipo I de forma precoz. La presencia de diabetes y un mayor requerimiento de glucosa para obtener una adecuada UF sugieren que la glucosa a ambos lados de la membrana peritoneal podrían ser las responsables. Los mecanismos de este alto requerimiento aún no están aclarados. El estudio de mayores cohortes de pacientes con fallo de UF precoz nos permitirán explorar mejor los mecanismos patogénicos primarios.

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REVIEWS AND ORIGINAL ARTICLES

RISK FACTORS RESPONSIBLE FOR ULTRAFILTRATION FAILURE IN EARLY STAGES OF PERITONEAL DIALYSIS

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♦ ***Objective:*** **To define risk factors for ultrafiltration failure (UFF) during early stages of peritoneal dialysis (PD).**
♦ ***Design:*** **Retrospective analysis of a group of patients whose peritoneal function was prospectively followed.**
♦ ***Setting:*** **A tertiary-care public university hospital.**
♦ ***Patients:*** **Nineteen of 90 long-term PD patients required a peritoneal resting period to recover UF capacity: 8 had this requirement before the third year on PD (early, EUFF group) and 11 had a late requirement (LUFF group). The remaining 71 patients, those with stable peritoneal function over time, constituted the control group.**
♦ ***Main Outcome Measures:*** **Peritoneal UF capacity under standard conditions (monthly) and small solute peritoneal transport (yearly).**
♦ ***Results:*** **None of the conditions appearing at the start of PD or during the observation period could be definitely identified as the cause of UFF. There were no differences in characteristics between the EUFF group and the other two groups, except for the higher prevalence of diabetes in the EUFF group. Residual renal function (RRF) declined in all three groups during the first 2 years, with rapid loss during the third year in the EUFF group. This rapid loss in RRF was coincident with UFF. Peritoneal solute and water transport at baseline was similar in the three groups. After 2 years on PD, individuals in the EUFF group showed a significantly lower UF and higher creatinine mass transfer coefficient values than those in the LUFF group. Diabetic patients in the control group showed remarkable stability in UF capacity over time. During the second year on PD, requirement for increases in dialysate glucose concentration was 3.4 ± 0.5% in the LUFF group, but as high as 25.5 ± 24.2% in the EUFF group. The accumulated days of active peritonitis (APID, days with cloudy effluent) were similar for the three groups after 1, 2, and 3 years on PD. Interestingly, diabetic patients in the control group showed an APID index significantly lower than the overall EUFF group. Diabetics in the control group also had significantly lower APID versus nondiabetics in the control group ($p = 0.016$).**
♦ ***Conclusions:*** **Our findings suggest that certain patients develop early UFF type I. Diabetic state and a higher glucose requirement to obtain adequate UF suggest that glucose on both sides of the peritoneal membrane could be responsible. The mechanisms for this higher requirement remain to be elucidated. The identification of a larger cohort of these early UFF patients should lead to a better exploration of the primary pathogenic mechanisms.**

KEY WORDS: Peritoneal ultrafiltration capacity; ultrafiltration failure type I; peritoneal survival; peritoneal tolerance to glucose.

We recently showed that 20% of long-term peritoneal dialysis (PD) patients develop ultrafiltration failure (UFF) with a high peritoneal solute transport state (UFF type I) (1). Nineteen of 90 patients studied (49 of whom had been on PD for more than 5 years) showed fluid volume overload and required a peritoneal resting period to recover UF capacity (1,2). All these 19 patients had started PD in conditions similar to those who never developed fluid overload secondary to UFF type I. None of the conditions appearing during the observation period could be definitely identified as the cause of the peritoneal changes leading to UFF.

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The importance of a high peritoneal transport state among PD patients is reflected by the fact that medium-term survival may be lower compared to normal-to-low transporter patients (3,4). Reasons include fluid volume overload, poor blood pressure control, and metabolic abnormalities induced by high peritoneal glucose overload (3,4).

Unfortunately, no conditions other than recurrent and/or severe peritonitis have been related to the process. Davies *et al.* (3) suggested that a premature use of larger amounts of intraperitoneal glucose is associated with this disorder.

The exact prevalence of this peritoneal functional abnormality is not clearly defined. Only our study (1) has distinguished between early and late forms of UFF.

Earlier presentation of this disorder is important in that it imposes limitations on the use of PD immediately after initiation; it represents a true state of peritoneal intolerance to dialysis.

The main aim of the present paper was to define, by studying the medical records and characteristics of 8 patients who developed a premature high peritoneal transport state, risk factors for the development of UFF during the early stages of PD (before the third year). A second objective was to propose strategies to prevent this condition.

PATIENTS AND METHODS

The study involved 261 patients from our PD program, including the 90 patients analyzed in an earlier publication (1), who had had at least two annual peritoneal function evaluations. A new analysis was performed of the particular conditions and characteristics of groups formed according to whether a 4-week peritoneal rest period for UFF was required (19 patients) and when they required it, or not required (71 patients) (1,2,5). The group of 19 patients was consequently divided into two smaller groups: 8 patients who required peritoneal resting before their third year on PD (early, EUFF group), and 11 patients with a late requirement for peritoneal resting (LUFF group). In practical terms, we established UFF by the need for peritoneal rest. Immediately prior to the peritoneal rest, all these patients showed serious problems with fluid volume control (i.e., volume-dependent hypertension, edema, congestive cardiac failure, and acute pulmonary edema). These clinical findings were accompanied by an abnormally low peritoneal UF capacity (net UF < 400 - 500 mL with 3.86% dextrose, then 100 mL with 1.36% dextrose and a high peritoneal creatinine transport [mass transfer coefficient (MTC) > 12 mL/minute], which allowed the diagnosis of UFF type I (1,2,5,6). The remaining 71 patients, who showed remarkable peritoneal functional stability over time, constituted the control group (1). All comparisons between the three groups (EUFF group vs LUFF group vs those without UFF as controls) were made in order to define the possible reasons for early UFF.

The peritoneal resting period consisted of 4 weeks on hemodialysis. During this period, 200 mL 1.36% glucose dialysis solution containing 35 mg (3500 IU) sodium heparin was infused twice per week into the peritoneal cavity.

To confirm that this was the only population that behaved in this way, we reviewed the peritoneal records of the other 171 patients who had been on PD for at least 1 year but not included in our previous three studies (1,5,6). None of these 171 patients had UFF necessitating a peritoneal resting period.

During early PD stages, patients usually maintained some residual renal function (RRF), thus achieving adequate volume control. This in fact differentiates our early UFF group from patients without UFF, as the former could not maintain adequate fluid volume status, although some degree of renal function was present (see below).

None of the patients included in this study had suffered severe peritonitis during the first 3 years on PD and all used glucose as osmotic agent.

Peritoneal function was evaluated by measuring the daily net UF capacity, under standard glucose concentration and dwell time conditions, and determining the peritoneal MTC for small molecules (1,6). Since the beginning of our PD program (1980), it has been our usual practice to monitor peritoneal UF capacity monthly by averaging the daily data recorded on each patient's sheet. In this way, we have been able to calculate net UF under similar glucose and dwell-time standard conditions (three daily exchanges, one 3.86% dextrose, one 1.36% dextrose, and one nocturnal 1.36% dextrose exchange). In recent times when 1.36% dextrose was substituted for 2.27% dextrose, we occasionally have had to extrapolate data to maintain comparable calculations. Details of the calculations and extrapolation techniques are given in previous papers (1,2, 5,6).

We also studied RRF (yearly), serum albumin (yearly average from bimonthly evaluations), peritonitis episodes, accumulated days of peritoneal inflammation determined by cloudy effluent (APID) (6), and accumulated peritoneal glucose load.

Statistical analysis was performed using paired and nonpaired Student's *t*-tests for parametric data and Mann-Whitney and Wilcoxon tests for nonparametric data. A p value less than 0.05 was considered significant.

RESULTS

There were no differences in demographic characteristics between the EUFF group and the other two

groups, except for the higher prevalence of diabetics (50% in the EUFF group, 27% in the LUFF group, and 23% in the control group; $p < 0.05$). Mortality was similar in the three groups. Transfer to hemodialysis was more frequent among patients in the EUFF group than the LUFF group (37% vs 27%, $p < 0.05$).

The RRF follow-up during the first 3 years is shown in Figure 1. Values were similar at the start of PD, with a similar decline appearing in all three groups during the first 2 years. The EUFF group showed, however, a significantly higher rate of loss of renal function versus the other two groups during the third year. This phenomenon was coincident with the peritoneal rest requirement. It did not antecede UFF.

Peritoneal solute and water transport at baseline were similar for the three groups. The values for UF and creatinine MTC are shown in Table 1. From the start of PD until the 12th month, the control and LUFF groups showed a trend toward an increase in UF, whereas the EUFF group showed no differences. The differences reached statistical significance during the second year on PD, when the EUFF group showed a remarkably lower UF capacity than the other two groups (Figure 2). This trend increased during the third year, when peritoneal rest was required.

Creatinine MTC values showed a similar but opposite tendency (Table 1). At the end of the second year, the EUFF group showed higher values than the LUFF and control groups. In addition, the usual trend to reduction of creatinine MTC values between baseline and the first year, observed in the control and LUFF groups, was not seen in the EUFF group. In summary, at the end of the second PD year, the EUFF group displayed a 50% reduction in UF and a 65% increase in creatinine transport capacities versus baseline values.

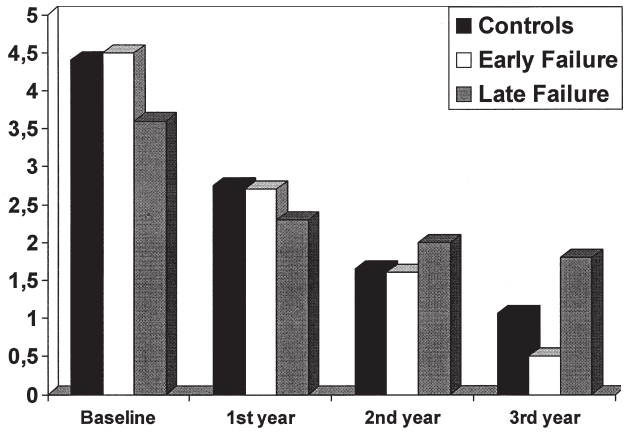

Figure 1 — Mean residual renal creatinine clearance (mL/min) was followed up during the first 3 peritoneal dialysis years in three groups of patients: early (before the third year) ultrafiltration failure (UFF) patients, late UFF patients, and control patients (mean ± SD).

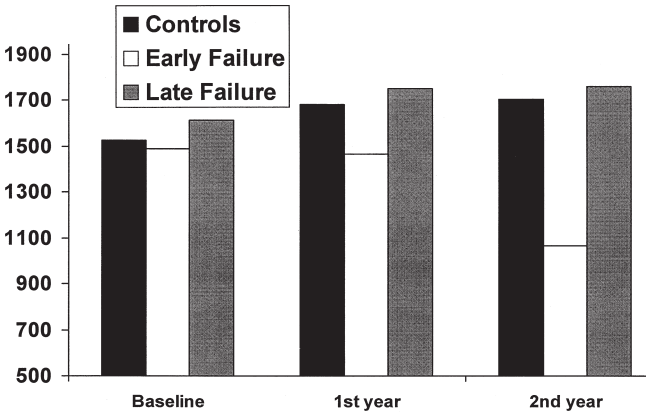

Figure 2 — Standardized mean daily ultrafiltration (UF) values are presented for three groups, early (before the third year) UF failure (UFF) patients, late UFF patients, and control patients, at baseline and during the first 2 peritoneal dialysis years. The decrease in UF for the early UFF group at the second year is statistically significant versus the earlier values ($p = 0.0097$).

Since a higher prevalence of diabetics was observed in the EUFF group, and to determine whether diabetes itself might lead to premature UFF, we examined the follow-up of the 15 diabetic patients in the control group. These patients showed a remarkable stability in UF capacity from baseline until the fourth year (1553 ± 611 mL/day vs 1665 ± 645 mL/day, NS). Creatinine peritoneal transport did not change significantly during the same period. The only change found was a decrease in the urea MTC/creatinine MTC ratio (3.13 ± 1.65 vs 1.87 ± 0.35, $p < 0.05$), a change not present in nondiabetic controls.

The peritoneal glucose load was calculated from the records of the two UFF groups; Table 2 shows the comparative values. The calculations were based on the proportion of the different glucose concentrations used every day.

Whereas glucose loads were similar during the first year, it was significantly higher in the EUFF group during the second year. This group also showed a higher glucose requirement during the second versus the first year. The increment of peritoneal glucose load between the first and second year was 3.4% ± 0.5% in the LUFF group and 25.5% ± 24.2% in the EUFF group ($p = 0.04$).

The APID (6), representing peritonitis accumulation over time, was similar for the three groups after 1, 2, and 3 years on PD. No particular individual was identified as having more severe peritonitis episodes (in terms of duration) in the EUFF group. The number of episodes after 3 years on PD was also similar for the EUFF and the LUFF groups. Interestingly, diabetic patients in the control group showed an APID remarkably lower (1.62 ± 2.91) than the overall EUFF group (5.4 ± 3.4 days, $p = 0.009$) and the nondiabetic

TABLE 1
Ultrafiltration Capacity and Peritoneal Creatinine Mass Transfer Coefficients (MTC) in Early (Before the Third Peritoneal Dialysis Year) Ultrafiltration Failure (EUFF), Late UFF (LUFF), and Control Groups (Values expressed as mean ± SD)

	Controls	EUFF Group	LUFf Group
Ultrafiltration (mL/day)			
Baseline	1524±621	1487±546	1615±533
First year	1682±549	1468±487	1750±501
Second year	1707±591	1065±438	1759±559
p Value[a]	NS	0.0097	NS
Creatinine-MTC (mL/min)			
Baseline	10.4±5.8	10.1±2.8	9.1±2.8
First year	9.7±3.3	10.0±2.8	8.5±2.4
Second year	9.1±3.3	16.7±4.9	9.0±3.7
p Value[a]	NS	0.05	NS

[a] Second year versus baseline and first year.

TABLE 2
Peritoneal Glucose Load (in kilograms) over the First Two Years in Early (Before the Third Peritoneal Dialysis Year) Ultrafiltration Failure (EUFF) and Late UFF (LUFF) Groups

	EUFF Group	LUFF Group
Glucose		
First year	56.9±13[a]	52.5±9.1[b,c]
Second year	70.1±14	53.9±8.9[d]
Glucose accumulated after 2 years	127±24	106±17[d]
Increment of peritoneally administered glucose (second vs first years)	25.5%±24.2%	3.4%±12.5%[d]

[a] $p = 0.01$, EUFF group, first year versus second year.
[b] Difference not significant, EUFF group first year versus LUFF group first year.
[c] Difference not significant, LUFF group, first year versus second year.
[d] $p=0.04$, EUFF group second year versus LUFF group second year.

controls (4.3 ± 6 days, $p = 0.016$). In other words, for a diabetic to be maintained in the group with stable UF rates, it seems that the patient is required to remain almost free of peritonitis. A minimal incidence seems to lead them to the EUFF group.

Serum albumin was examined in both UFF groups. The rationale for this relationship was the possibility that lower serum albumin levels might represent an acute phase reactant, affecting peritoneal vasculature. A potential peritoneal vasodilatation might increase creatinine and glucose transports. Nonetheless, no significant differences between albumin levels in the EUFF and LUFF groups were found at any observation time; serum albumin was in fact slightly higher in the EUFF group during the third year (mean 4.01 g/dL vs 3.67 g/dL, $p = 0.056$). Values were similar for the other years analyzed.

DISCUSSION

These results suggest some risk factors that can be identified in patients who develop early UFF with an increase in solute transport after 12 - 36 months on PD. Peritoneal changes related to diabetes mellitus and the induction of peritoneal diabetiform changes by glucose-containing dialysate may be involved. This could be called "inherent UFF," to be distinguished from that developed by PD patients over time, as both a consequence of PD itself or the cumulative effects of peritonitis on the peritoneum.

In a recently published Forum (7), Krediet defines UFF type I as an UF capacity lower than 400 mL and high transport of creatinine after a 4-hour dwell with 3.86% glucose. When UFF type I is confirmed, a month of peritoneal resting is suggested. Krediet recommends that prevention of UFF not due to peritonitis (most of the present cases) requires the use of more biocompatible solutions from the start of PD. The important message conveyed by Krediet is that glucose in dialysate is considered the main culprit responsible for peritoneal membrane damage (7). Nonetheless, several questions remain to be answered.

Two important unsolved problems formulated in the Forum (7) are whether diabetes is involved in the

development of peritoneal membrane failure, and whether the results of the peritoneal equilibration test (PET) at the start of dialysis identify patients at risk for UFF.

Our present data show that the prevalence of diabetes is higher among patients with early membrane failure than among stable patients, although no remarkable functional differences (based on PET or MTCs) are demonstrated at the start of PD. Also, we believe that diabetic patients are particularly sensitive to developing UFF after suffering peritonitis, even if appropriately cured, which may have no negative consequences in nondiabetics. This can be deduced from the lower mean APID in control diabetics (mean 1.62 days) compared to the higher value in the EUFF group (mean 5.4 days), in which 50% were diabetics. In contrast, although nondiabetic controls presented similar APID values (4.3 days), they remained stable over time. This might suggest that the diabetic peritoneum is more sensitive to inflammatory processes.

With respect to the question on the existence or not of a diabetic peritoneopathy, diabetic patients starting PD show some previously described peritoneal permeability differences (8,9), which have been confirmed in the present study in a larger series of patients. Diabetics show different MTC values than nondiabetics: higher urea MTC and lower creatinine MTC (data not shown).

No other data at the start of PD can be identified as a risk factor for early UFF. The peritoneal functional response to PD during the first year is, however, suggestive of something different. In fact, during the first year, the EUFF group showed stable creatinine MTC and no increase in UF capacity compared to baseline values, whereas the other two groups showed tendencies to decreased creatinine MTC and increased UF capacity. This different behavior reaches its maximal expression after the 12th month. At this point, MTC and UF were absolutely different (Table 1). It seems that a patient whose UF capacity does not increase, and whose creatinine transport does not decrease, during the first 12 months compared to baseline values, is at risk for early membrane failure. In these patients, the use of progressively higher amounts of glucose during the second year culminated the vicious cycle leading to UFF. The loss in RRF (and consequent diminishing diuresis) was not the initiating cause of the peritoneal defect. This phenomenon coincided with, but did not precede, peritoneal membrane alteration and glucose overuse. It is not known whether activation of a systemic chronic inflammatory process generated at the peritoneum and involving renal tissue precipitated the loss of renal function. The possible defect in our study was to evaluate MTCs and RRF only on a yearly (and not more frequent) basis.

Diabetic changes on both sides of the peritoneal membrane (capillary–subendothelium and mesothelial–submesothelial layers) caused by diabetes and peritoneal glucose load respectively, may be the initiating and perpetuating factors for peritoneal neoangiogenesis. This is in fact the anatomical substrate of UFF type I (10). One possible mediator of this process is vascular endothelial growth factor (VEGF). A close relationship has been established between VEGF and diabetes in proliferative retinopathy (11,12). The link between dialysate glucose, peritoneal advanced glycosylation end-product formation, and VEGF production by peritoneal capillary endothelial cells seems to be an attractive hypothesis. To combat the neoangiogenic process, only avoidance of the principal cause, glucose, can be suggested at present. Alternative osmotic agents, such as amino acids and icodextrin, are now available to diminish the daily use of glucose-containing dialysate. We propose the prophylactic use of these agents, combined with glucose exchanges, in high-risk patients from the start of PD. In the future, other alternatives will be found to avoid glucose completely. Several recent studies confirm the utility and safety of amino acid and icodextrin solutions (13–15). In an animal model, amino acid solution has been demonstrated to be protective to mesothelial cells and the submesothelial space in comparison with glucose solutions (16). Evidently, the positive effects of these solutions on the peritoneum during medium- to long-term use remain to be determined. VEGF production by mesothelial cells has been recently demonstrated (17), and a link may exist between glucose, mesothelial cell VEGF production, and peritoneal neoangiogenesis.

Our data have not demonstrated severe systemic consequences of UFF, represented by a potentially higher mortality, as has been suggested (3,4). Díaz-Buxo *et al.* (18) recently showed the same lack of association. We proposed the maintenance of UFF patients on PD under appropriate care, including a peritoneal resting period whenever UFF appears. In a recent paper (19), the hypothesis that a high peritoneal transport rate might reflect a general state of chronic inflammation (as indicated by nonspecifically elevated serum levels of interleukin-1 and tumor necrosis factor) was not demonstrated in this particular population. Neither are our data of similar levels of serum albumin in the EUFF and LUFF groups suggestive of such a chronic inflammatory state.

In summary, our findings strongly suggest the existence of a premature peritoneal negative response to dialysate, manifested by early UFF type I. Hyperglycemia caused by diabetes and higher amounts of glucose in peritoneal dialysate could lead to diabetic changes on the blood and mesothelial sides of the

peritoneal membrane. The mechanisms by which glucose acts negatively in some diabetic and nondiabetic patients remains to be elucidated, as it appears to be an important but not the sole risk factor. The possibility exists to modify this negative trend with interventions such as the peritoneal rest or the use of dextrose-free solutions. The identification of these particular cohorts of patients, those with inherent UFF, should lead to a better-oriented exploration of the pathogenic mechanisms.

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Capítulo 2. Estructura de la membrana peritoneal al comienzo de la diálisis peritoneal

6.1.2.1.- "Diálisis peritoneal y transición epitelio-mesenquimal de las células mesoteliales"

Yáñez-Mó M, Lara-Pezzi E, Selgas R, Ramírez-Huesca M, Domínguez-Jiménez C, Jiménez-Heffernan JA, Aguilera A, Sánchez-Tomero JA, Bajo MA, Alvarez V, Castro MA, del Peso G, Cirujeda A, Gamallo C, Sánchez-Madrid F, López-Cabrera M.

New England Journal of Medicine 2003; 348: 403-413

Este trabajo responde al objetivo 2

Este estudio ha recibido el Premio de la Fundación Renal Iñigo Álvarez de Toledo (FRIAT) al mejor trabajo de investigación básica en el año 2003 (concedido a D. Manuel López Cabrera)

Diálisis peritoneal y transición epitelio-mesenquimal de las células mesoteliales.

El peritoneo de los pacientes tratados con diálisis peritoneal está expuesto a soluciones de diálisis bioincompatibles, lo que da lugar a la pérdida de la monocapa de células mesoteliales y fenómenos de fibrosis, provocando fallo de la capacidad de ultrafiltración. Los mecanismos que intervienen en este proceso no están bien aclarados.

Métodos: Se realizó un análisis fenotípico de las células mesoteliales aisladas del efluente peritoneal de pacientes tratados con DP continua ambulatoria, mediante citometría de flujo, inmunofluorescencia confocal, Western blotting, y PCR-transcriptasa inversa. Estas células fueron comparadas con células mesoteliales procedentes de omento y sometidas *in vitro* a distintos estímulos con el fin de reproducir la transdiferenciación que se observa durante el tratamiento con DP. Posteriormente, se confirmaron los resultados *in vivo* mediante un estudio inmunohistoquímico de biopsias peritoneales.

Resultados: Tras el inicio de DP, las células mesoteliales peritoneales sufren precozmente una transición de un fenotipo epitelial a un fenotipo mesenquimal. Durante ésta, pierden progresivamente su morfología epitelial, así como la expresión de citoqueratinas y E-cadherina, debido a la inducción del factor represor de transcripción *snail.* Además, las células mesoteliales adquieren capacidad migratoria mediante la inducción de sobre-regulación de la expresión de $\alpha_2$integrina. Los estudios *in vitro* revelaron que los factores iniciadores de la transdiferenciación mesotelial son el fenómeno de reparación tisular y las citoquinas profibróticas e inflamatorias. El análisis inmunohistoquímico de las

biopsias peritoneales de pacientes en tratamiento con DP mostró la expresión de marcadores de célula mesotelial, como la molécula de adhesión intercelular 1 (ICAM-1) y citoqueratinas, en células fibroblásticas localizadas en el estroma submesotelial. Esto sugiere que dichas células proceden de la conversión a nivel local de células mesoteliales.

Conclusiones: Las células mesoteliales tienen un papel activo en las alteraciones estructurales y funcionales del peritoneo sometido a DP. Los hallazgos encontrados sugieren potenciales dianas para el diseño de nuevas soluciones de diálisis y de marcadores para la monitorización de estos pacientes.

ORIGINAL ARTICLE

Peritoneal Dialysis and Epithelial-to-Mesenchymal Transition of Mesothelial Cells

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ABSTRACT

BACKGROUND

During continuous ambulatory peritoneal dialysis, the peritoneum is exposed to bioincompatible dialysis fluids that cause denudation of mesothelial cells and, ultimately, tissue fibrosis and failure of ultrafiltration. However, the mechanism of this process has yet to be elucidated.

METHODS

Mesothelial cells isolated from effluents in dialysis fluid from patients undergoing continuous ambulatory peritoneal dialysis were phenotypically characterized by flow cytometry, confocal immunofluorescence, Western blotting, and reverse-transcriptase polymerase chain reaction. These cells were compared with mesothelial cells from omentum and treated with various stimuli in vitro to mimic the transdifferentiation observed during continuous ambulatory peritoneal dialysis. Results were confirmed in vivo by immunohistochemical analysis performed on peritoneal-biopsy specimens.

RESULTS

Soon after dialysis is initiated, peritoneal mesothelial cells undergo a transition from an epithelial phenotype to a mesenchymal phenotype with a progressive loss of epithelial morphology and a decrease in the expression of cytokeratins and E-cadherin through an induction of the transcriptional repressor *snail.* Mesothelial cells also acquire a migratory phenotype with the up-regulation of expression of α_2 integrin. In vitro analyses point to wound repair and profibrotic and inflammatory cytokines as factors that initiate mesothelial transdifferentiation. Immunohistochemical studies of peritoneal-biopsy specimens from patients undergoing continuous ambulatory peritoneal dialysis demonstrate the expression of the mesothelial markers intercellular adhesion molecule 1 and cytokeratins in fibroblast-like cells entrapped in the stroma, suggesting that these cells stemmed from local conversion of mesothelial cells.

CONCLUSIONS

Our results suggest that mesothelial cells have an active role in the structural and functional alteration of the peritoneum during peritoneal dialysis. The findings suggest potential targets for the design of new dialysis solutions and markers for the monitoring of patients.

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Continuous ambulatory peritoneal dialysis is an alternative to hemodialysis for the treatment of end-stage renal disease.[1] The peritoneal membrane is lined with a monolayer of mesothelial cells that have some characteristics of epithelial cells, act as a permeability barrier, and secrete various substances involved in the regulation of peritoneal permeability and local host defense.[1,2] Unfortunately, long-term exposure to the hyperosmotic, hyperglycemic, and acidic solutions used in dialysis often causes low-grade, chronic inflammation of and injury to the peritoneum, which progressively becomes denuded of mesothelial cells and undergoes fibrosis.[1] Such structural alterations are considered to be the principal cause of failure of ultrafiltration, which affects up to 20 percent of patients undergoing continuous ambulatory peritoneal dialysis.[3] This functional decline of the peritoneum may be accelerated by recurrent or severe episodes of peritonitis or hemoperitoneum.[3,4]

The pathophysiology of peritoneal impairment during long-term continuous ambulatory peritoneal dialysis is not well understood. Peritoneal mesenchymal stem cells entrapped in the stroma have historically been considered to be the primary cells involved in the development of peritoneal fibrosis.[5] However, a possible direct involvement of mesothelial cells in this phenomenon has not been examined. In this context, cultured mesothelial cells have the capacity to change their morphologic features and produce extracellular-matrix components in response to a variety of stimuli.[6-12] In addition, treatment of mesothelial cells in vitro with mediums that have a high glucose concentration or with inflammatory cytokines induces the expression of transforming growth factor β (TGF-β)[13] and decreases the expression of E-cadherin.[14] The relevance of the profibrotic growth factor TGF-β[15] in the failure of ultrafiltration induced by continuous ambulatory peritoneal dialysis was recently underscored in a rat model in which the *TGF-β* gene was transduced to the peritoneum, where it was associated with a decrease in peritoneal function.[16]

In the present study, we demonstrate in vivo and ex vivo that mesothelial cells undergo a transition from an epithelial phenotype to a mesenchymal phenotype — a transition also called transdifferentiation — when they are subjected to peritoneal dialysis. Transdifferentiation is a complex and generally reversible process that starts with the disruption of intercellular junctions and loss of the apical–basolateral polarity typical of epithelial cells, with the cells then transformed into fibroblast-like cells with pseudopodial protrusions and increased migratory, invasive, and fibrogenic features.[17] Although transdifferentiation can be induced in most cultured epithelial cells with a wide variety of treatments, this process occurs in vivo only during embryonic development and in some pathologic processes such as wound healing and tumor progression.[17,18] The intercellular adhesion molecule E-cadherin appears to have a central role in the control of the epithelial-to-mesenchymal transition, since the loss of E-cadherin expression or function correlates with the ability of epithelial cells to adopt a mesenchymal migratory and invasive phenotype.[19,20] The transcription factor *snail* is a strong repressor of E-cadherin transcription and an inducer of transdifferentiation.[21-23] Thus, phenotypic changes of the mesothelial cells during continuous ambulatory peritoneal dialysis may be directly related to the failure of peritoneal membrane function.

METHODS

PATIENTS AND CELLS

Human mesothelial cells from effluent (mean [±SE] number of cells per bag, 25,569±2971) were obtained by centrifugation of dialysis fluid taken randomly from 54 clinically stable patients undergoing nocturnal exchanges with dialysis solutions containing 2.27 percent glucose and 1.25 to 1.75 mmol of calcium per liter. After 10 to 15 days, cultures reached confluence and were split (in a ratio of 1:2) two to three times. The morphologic features of cells in confluent cultures were compared and remained stable during the two to three cell passages. Eighty-five percent of the cultures were obtained before a first episode of peritonitis occurred. Of the 116 effluent cultures evaluated, 62 had cobblestone morphology, 28 contained transitional mesothelial cells, 20 contained fibroblast-like mesothelial cells, and 6 contained a mixed population of cells.

Omental mesothelial cells were obtained by digestion of samples of omentum from 30 patients who were not undergoing continuous ambulatory peritoneal dialysis but were undergoing unrelated abdominal surgery; the samples were digested with 0.05 percent trypsin and 0.02 percent EDTA. Omental fibroblasts were obtained from three different samples of omentum by extensive treatment with trypsin after the removal of mesothelial cells (three 20-minute rounds of exposure to trypsin). All cells

were cultured in Earle's M199 medium, 20 percent fetal-calf serum, 50 U of penicillin per milliliter, 50 μg of streptomycin per milliliter, and 2 percent Biogro-2 (containing insulin, transferrin, ethanolamine, and putrescine) (Biological Industries). For the experiments, cells were seeded on films of 50 μg of collagen I per milliliter without Biogro-2. TGF-β1 and interleukin-1β were purchased (R&D), and the doses used were in the range of those detected in peritoneal-dialysis fluids in the presence of peritonitis[24] and were similar to those used in previous studies.[9] The study was approved by the ethics committee of Hospital Universitario de la Princesa in Madrid, and oral informed consent was obtained from all donors.

ANTIBODIES

Monoclonal antibodies against CD151 (LIA1/1), CD9 (VJ1/20), α_3 integrin (VJ1/18), β_1 integrin (TS2/16), and α_2 integrin (TEA1/41) have been described elsewhere.[25] We also used monoclonal antibody against intercellular adhesion molecule 1 (ICAM-1) (HU5/3, provided by Dr. F.W. Luscinskas, Brigham and Women's Hospital, Boston); rabbit polyclonal antibodies against α_2 integrin and α_3 integrin (provided by Dr. G. Tarone, University of Turin, Turin, Italy); monoclonal antibody against E-cadherin (Calbiochem); antibodies against vimentin, α tubulin, and pancytokeratin (Sigma); and monoclonal antibody against ICAM-1 (Santa Cruz Biotechnology).

FLOW CYTOMETRY, IMMUNOHISTOCHEMICAL ANALYSIS, IMMUNOFLUORESCENCE STUDIES, AND CONFOCAL MICROSCOPY

Flow cytometry and immunofluorescence studies were performed as described previously.[25] Immunohistochemical studies were performed with a streptavidin–biotin method (Dako LSAB-2 Kit, Dako) on paraffin-embedded peritoneal-tissue samples from 17 patients undergoing continuous ambulatory peritoneal dialysis and 8 control patients. All patients who underwent biopsy gave written informed consent. Diaminobenzidine and fast red were used as chromogens for visualization.

WESTERN BLOTTING

Monolayers of mesothelial cells were lysed in RIPA buffer, and equivalent amounts of protein were resolved by sodium dodecyl sulfate–polyacrylamide-gel electrophoresis and Western blotting as described previously.[25] Imaging was performed with an LAS-1000 CCD camera (Fujifilm), and signals were quantified with Image Gauge software (version 3.46, Fujifilm).

REVERSE-TRANSCRIPTASE POLYMERASE CHAIN REACTION

Mesothelial RNA was extracted with the use of a reagent (RNAwiz, Ambion). The complementary DNA was obtained from 1 μg of total RNA with the use of a kit (Applied Biosystems). Amplification of *snail* was performed for 40 cycles (40 seconds at 95°C, 30 seconds at 53°C, and 1 minute at 72°C) with the use of primer 1 (5′CACATCCTTCTCACTGCCATG3′) and primer 2 (5′GCATCTAAACTCTAGTCTGC3′). For nested reverse-transcriptase–polymerase-chain-reaction (RT-PCR) analysis of *snail*, a 30-cycle reaction was performed under the same conditions, and a 1:50 dilution of the product of the reaction was amplified for 20 cycles (40 seconds at 95°C, 30 seconds at 60°C, and 1 minute at 70°C) with primer 1 and primer 3 (5′CCTGAGTGGGGTGGGAGCTTCC3′).[22] PCR analysis of E-cadherin was carried out for 32 cycles as described previously.[22]

MIGRATION ASSAYS

Assays of chemotaxis and haptotaxis (migration toward matrix proteins) were performed in polycarbonate transwell inserts (5-μm pore [Costar]), some of which were coated at the bottom with 10 μg of collagen I or laminin-5 per milliliter,[26] as previously described.[27]

TIME-LAPSE VIDEOMICROSCOPY

Videomicroscopical analysis was performed with the use of an inverted microscope equipped with a video camera (SSC-M350CE CCD, Sony) coupled to a time-lapse videocassette recorder (SVT-5000P, Sony). Mesothelial cells from omentum were subjected to mechanical injury with an adapted cell scraper approximately 1500 μm in width and recorded for two to three days until the "wound" closed in an incubator that maintained the sample at 37°C in an environment containing 5 percent carbon dioxide. Digitalization of the images was performed with the use of Optimas software (version 5.2, Bioscan).

RESULTS

MORPHOLOGIC CHANGES IN MESOTHELIAL CELLS DURING PERITONEAL DIALYSIS

Mesothelial-cell cultures from effluents from patients undergoing continuous ambulatory peritoneal dialysis had markedly varied morphologic fea-

tures, ranging from a cobblestone-like appearance similar to that of mesothelium derived from omentum to fibroblast-like cells or mixed cell populations (Fig. 1A). The prevalence of nonepithelioid cells appeared to be related both to the duration of continuous ambulatory peritoneal dialysis in each patient (Fig. 1B) and to whether and when hemoperitoneum or peritonitis had occurred. Fibroblast-like mesothelial cells appeared sporadically in samples in which hemorrhage or infiltrating lymphoid cells were present in the effluent, and a reversion to cobblestone or transitional phenotype was evident (in eight of eight cases) when cultures from the same patient were analyzed after the episode of peritonitis or hemoperitoneum had resolved (Fig. 1C).

To determine the nature of cells derived from effluent, the expression of cytokeratins, as typical epithelial markers, and of ICAM-1, which is constitutively expressed on mesothelial cells,[28] was analyzed. A high level of expression of cytokeratins was observed in omental mesothelial cells and effluent cells with cobblestone-like appearance (Fig. 1A). Cells derived from effluent showed a progressive reduction in the expression of cytokeratins, although even in cultures of fibroblast-like cells, a small population of positive cells was maintained

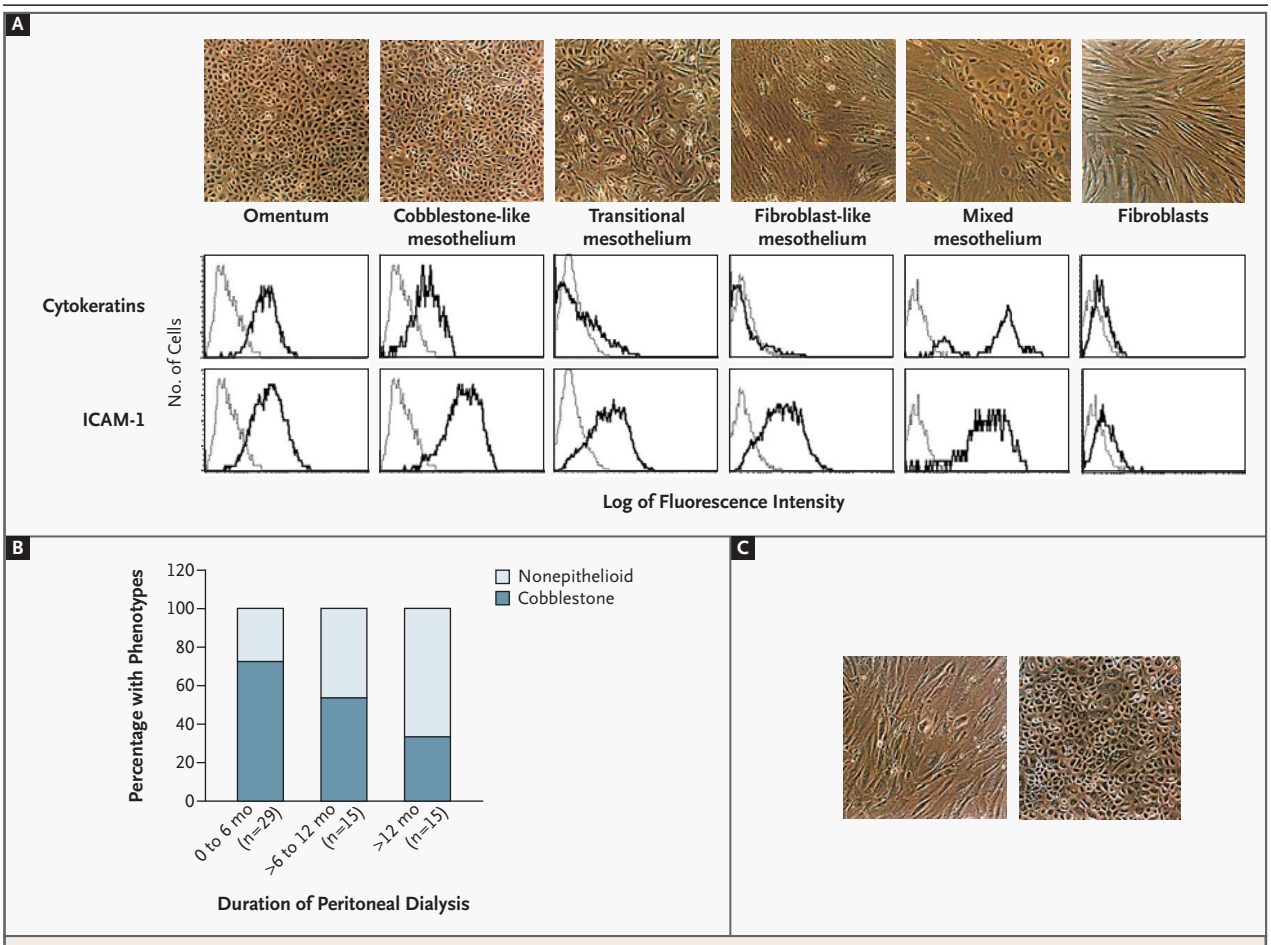

Figure 1. Morphologic Changes in Mesothelial Cells during Peritoneal Dialysis.

Panel A shows photomicrographs (×200) of confluent monolayers of the various cell preparations used in the study. Below the photomicrographs are flow-cytometric histograms of the various types of cells stained with monoclonal antibodies against cytokeratin or intercellular adhesion molecule 1 (ICAM-1). The gray lines represent negative controls. Panel B shows the relation between morphologic changes in mesothelial cultures and the duration of peritoneal dialysis. The mean (±SE) duration of peritoneal dialysis was 7±1 months among patients with cobblestone-like cultures and 13±2 months among those with nonepithelioid cultures (P=0.01 by Student's t-test). In addition, a test of linear tendency gave a χ^2 value of 6.193, with P=0.01 for the association of morphology with duration of dialysis. Panel C shows photomicrographs (×200) of mesothelial cells from effluent from the same patient during the course of an episode of hemoperitoneum (left-hand panel) and two months after remission of the pathologic process (right-hand panel).

(Fig. 2). Two peaks of keratin expression were observed in mixed cultures, whereas keratin expression was absent from fibroblasts from omentum. However, all cells from effluent, even in mixed cultures, had a high level of homogeneous expression of ICAM-1 that was independent of their morphologic features. In contrast, ICAM-1 expression was negligible on fibroblasts taken directly from both omentum and skin (Fig. 1A), supporting the theory that fibroblastoid cells in effluent have a mesothelial origin and their presence is not the result of contamination by fibroblasts.

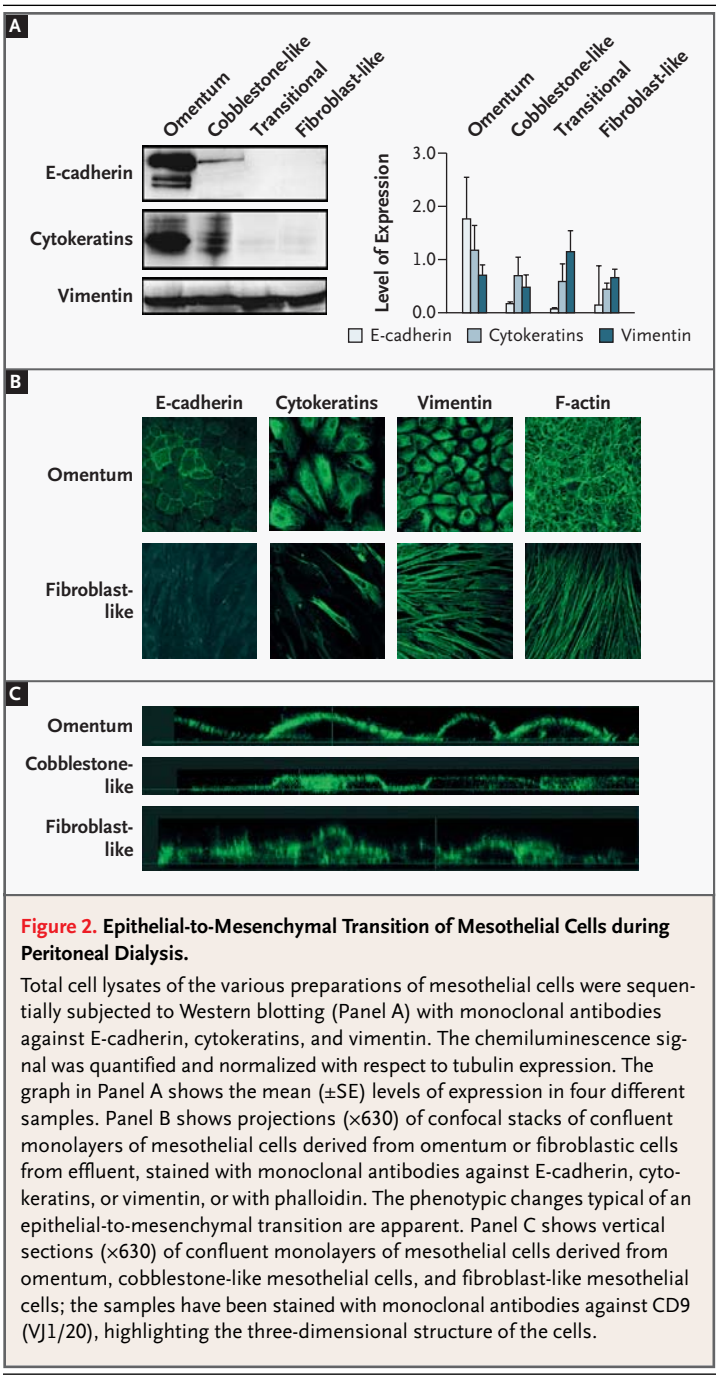

Figure 2. Epithelial-to-Mesenchymal Transition of Mesothelial Cells during Peritoneal Dialysis.

Total cell lysates of the various preparations of mesothelial cells were sequentially subjected to Western blotting (Panel A) with monoclonal antibodies against E-cadherin, cytokeratins, and vimentin. The chemiluminescence signal was quantified and normalized with respect to tubulin expression. The graph in Panel A shows the mean (±SE) levels of expression in four different samples. Panel B shows projections (×630) of confocal stacks of confluent monolayers of mesothelial cells derived from omentum or fibroblastic cells from effluent, stained with monoclonal antibodies against E-cadherin, cytokeratins, or vimentin, or with phalloidin. The phenotypic changes typical of an epithelial-to-mesenchymal transition are apparent. Panel C shows vertical sections (×630) of confluent monolayers of mesothelial cells derived from omentum, cobblestone-like mesothelial cells, and fibroblast-like mesothelial cells; the samples have been stained with monoclonal antibodies against CD9 (VJ1/20), highlighting the three-dimensional structure of the cells.

EPITHELIAL-TO-MESENCHYMAL TRANSITION IN VIVO

The morphologic changes and down-regulation of keratin in mesothelial cells derived from effluent could be indicative of an epithelial-to-mesenchymal transition.[17] We analyzed the expression of E-cadherin and the intermediate filament proteins cytokeratin and vimentin by Western blotting, as markers of transdifferentiation. There was a markedly lower level of E-cadherin expression in cobblestone and nonepithelioid mesothelial cultures than in omental cultures (Fig. 2A). The expression of cytokeratins (Fig. 2A) paralleled that of E-cadherin, whereas there was greater vimentin expression in nonepithelioid mesothelial cultures.

Confocal immunofluorescence microscopy demonstrated the loss of intercellular E-cadherin and the reorganization of the actin cytoskeleton from the cortical band typical of epithelial cells to fibroblastic stress fibers (Fig. 2B). Cytokeratin was replaced by vimentin, although some fibroblastoid mesothelial cells were still positive for keratin. Preparations stained for CD9 (Fig. 2C), which is expressed at apical microvilli and intercellular contacts,[29] showed a gradual loss of cuboid epithelial morphologic features, which was already evident in cobblestone-like mesothelial cells that were half as high as omental mesothelium in confocal vertical sections. Fibroblast-like mesothelial cells lost contact inhibition and frequently piled up on one another.

EFFECTS OF MECHANICAL INJURY, TGF-β1, AND INTERLEUKIN-1β

The behavior of mesothelial cells during in vitro wound healing was dynamically assessed after the mechanical denudation of confluent monolayers of cells derived from omentum. Mechanical stimulus was sufficient to induce migration of mesothelial cells, and migrating cells underwent a transitional transdifferentiation in which a mesenchymal morphology reverted to an epithelial aspect only after the monolayer was restored (Fig. 3A, 3B, 3C, 3D, 3E, and 3F). This effect was confined to cells at the edge of the wound and neighboring areas, whereas cells at a distance from the wound were not modi-

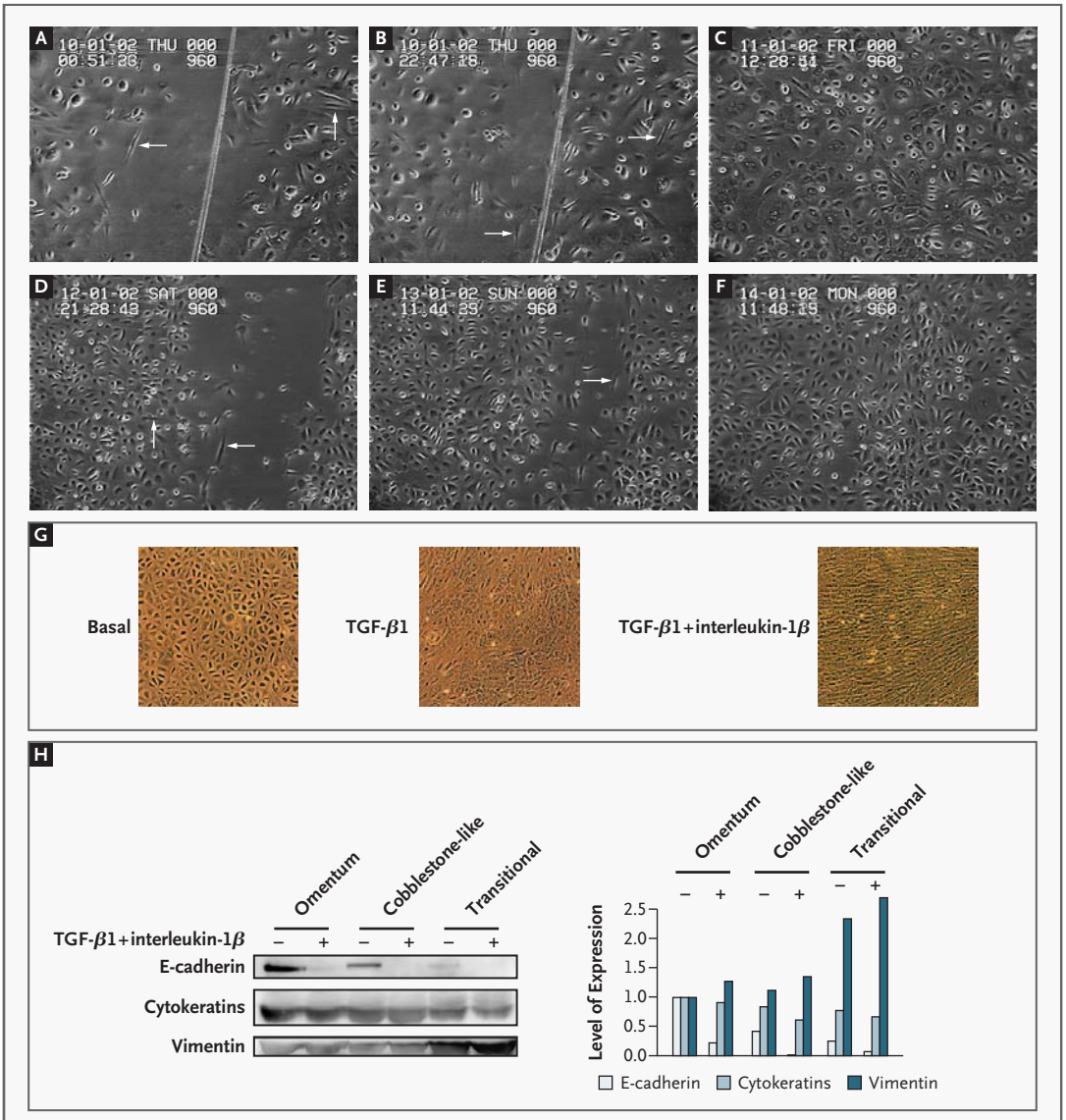

Figure 3. Mesothelial Transdifferentiation in Vitro, Induced by Mechanical Injury, Transforming Growth Factor β1 and Interleukin-1β.

Confluent monolayers of mesothelial cells from omentum were mechanically wounded and allowed to migrate for two to three days. Representative photomicrographs of the video sequences from two independent experiments are shown (one in Panels A, B, and C; the other in Panels D, E, and F; all ×200). Mesothelial cells with fibroblastic appearance are observed both in the front layer of migrating cells and behind, in the monolayer (arrows). This transition is only local, and cells distant from the wound maintain their epithelioid morphologic features (Panel C). Transdifferentiation is also reversed once the wound is repaired (Panel F). Panel G shows photomicrographs (×200) of mesothelial cells derived from omentum, some of which were left untreated and some of which were treated with 0.5 ng of transforming growth factor β1 (TGF-β1) per milliliter, in some cases in combination with 2 ng of interleukin-1β per milliliter, for 48 hours. Total cell lysates of the various preparations of mesothelial cells, some of which were treated for 48 hours with 0.5 ng of TGF-β1 per milliliter and 2 ng of interleukin-1β per milliliter, were sequentially subjected to Western blotting (Panel H) with monoclonal antibodies against E-cadherin, cytokeratins, vimentin, and tubulin. The chemiluminescence signal was quantified and normalized with respect to tubulin expression and was related to the levels of expression of untreated omental cells in an experiment that was representative of the three that were performed.

fied, reinforcing the theory that the mechanical stimulus was sufficient to induce transdifferentiation. Complete time-lapse video sequences appear in Supplementary Appendix 1 (available with the full text of this article at http://www.nejm.org).

To determine whether TGF-β1 and interleukin-1β, two cytokines detected in effluents from patients undergoing continuous ambulatory peritoneal dialysis primarily during episodes of peritonitis,[24] could reproduce the phenotypic changes observed ex vivo, cultured mesothelial cells derived from omentum were treated with TGF-β1 alone or in combination with interleukin-1β. An additive morphologic effect of both stimuli could be observed (Fig. 3G). E-cadherin expression was almost completely abolished (Fig. 3H), and its localization at intercellular junctions could hardly be detected by immunofluorescence. Cytokeratin expression was also diminished, and an additive effect with interleukin-1β was observed. In contrast, these treatments were associated with an increment in vimentin expression.

EXPRESSION OF *snail* IN MESOTHELIAL CELLS UNDERGOING EPITHELIAL-TO-MESENCHYMAL TRANSITION

Recently, a transcription factor called *snail* has been described as a potent repressor of E-cadherin expression and an inducer of epithelial-to-mesenchymal transition.[21-23] To determine whether *snail* expression was associated with the phenotypic changes observed in the cells of the peritoneal membrane in patients undergoing continuous ambulatory peritoneal dialysis, RT-PCR analysis was used to estimate the expression of this transcription factor, as well as that of E-cadherin, in mesothelial cells derived from effluent and omentum (Fig. 4A). No *snail* messenger RNA (mRNA) signal was detected in omental cells, whereas a progressive increase in the expression of *snail* mRNA was observed in effluent preparations as the process of transdifferentiation progressed. A dramatic down-regulation of expression of E-cadherin mRNA was already apparent in effluent cells that had a cobblestone appearance, a finding consistent with the decrease in expression of E-cadherin protein (Fig. 2A).

Stimulation of cultured mesothelial cells with TGF-β1 plus interleukin-1β revealed a rapid and transient induction of *snail* mRNA. E-cadherin mRNA was decreased by the time of the first observation and remained almost undetectable even af-

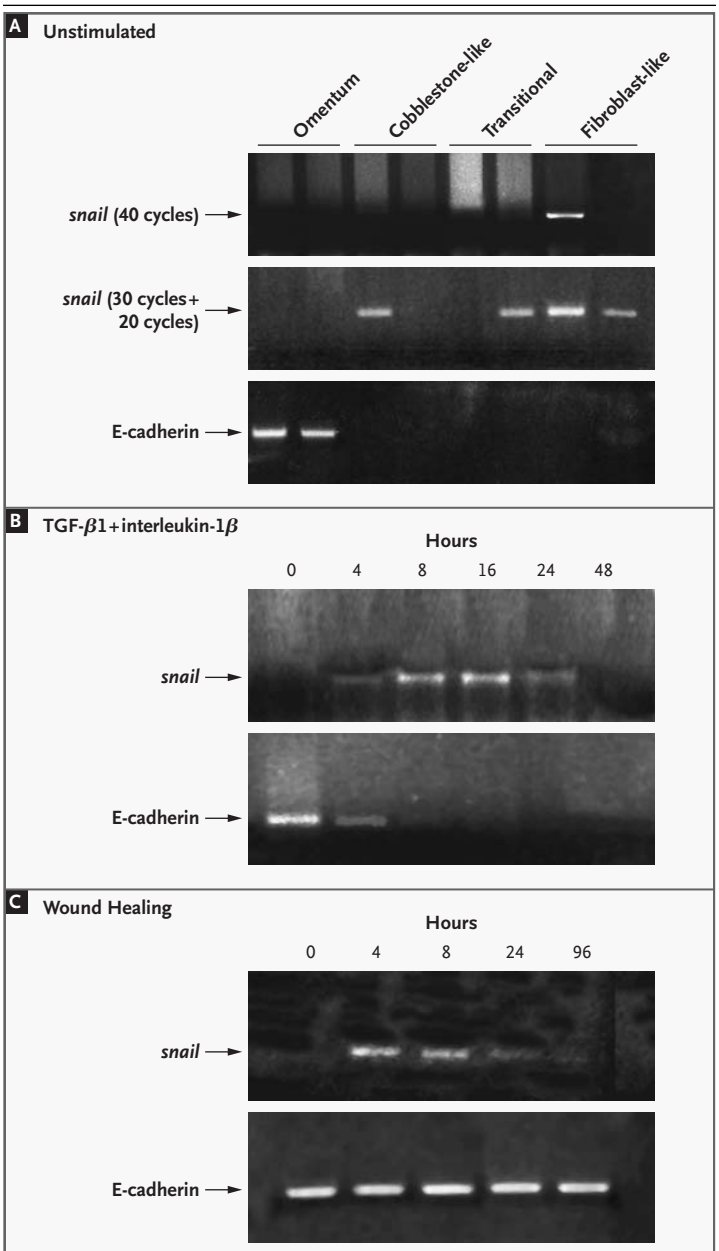

Figure 4. Transdifferentiation of Mesothelial Cells and Early Expression of the *snail* Transcription Factor.

Cells obtained from human omentum and effluents from peritoneal dialysis (two samples per type of transdifferentiated cell) were analyzed for *snail* and E-cadherin messenger RNA (mRNA) expression (Panel A) by RT-PCR. Omental cells were stimulated with 0.5 ng of transforming growth factor β1 (TGF-β1) per milliliter and 2 ng of interleukin-1β per milliliter at different times, and *snail* and E-cadherin mRNA expression was analyzed by RT-PCR (Panel B). Monolayers of omental cells were wounded and allowed to migrate, and the expression of *snail* and E-cadherin mRNA was analyzed by PCR at different times (Panel C).

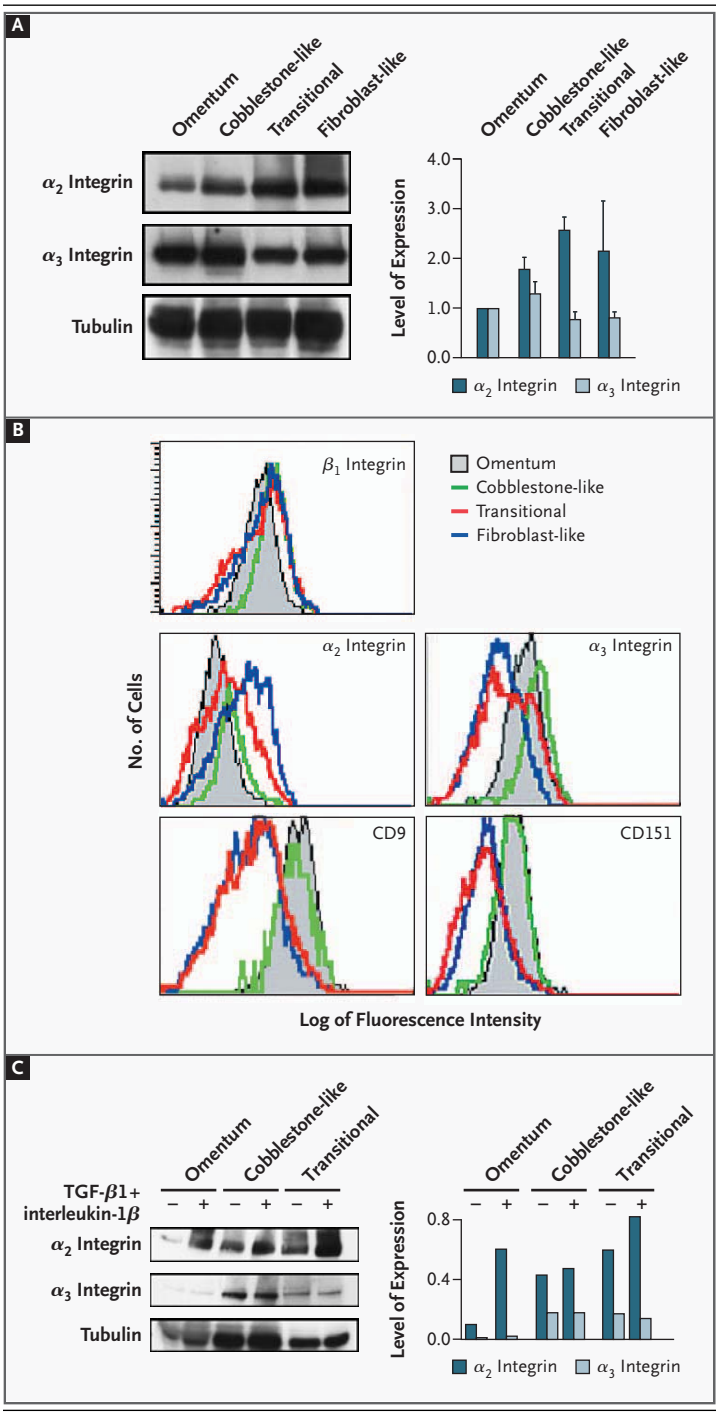

Figure 5. Up-Regulation of α_2 Integrin Expression and Down-Regulation of Expression of Tetraspanins through the Process of Mesothelial Transdifferentiation.

Total cell lysates of the various preparations of mesothelial cells were sequentially subjected to Western blotting with antibodies against α_2 integrin or α_3 integrin and monoclonal antibodies against tubulin (Panel A). The chemiluminescence signal was quantified, normalized with respect to tubulin expression, and related to the levels of expression in omental cells. The graph shows the mean (±SE) levels of expression in four different samples. Panel B shows flow-cytometric histograms of the various preparations of cells stained for β_1 integrin, α_2 integrin, α_3 integrin, CD9, or CD151. Total cell lysates of the various preparations of mesothelial cells, some of which were treated for 48 hours with 0.5 ng of transforming growth factor β1 (TGF-β1) per milliliter and 2 ng of interleukin-1β per milliliter, were sequentially subjected to Western blotting with antibodies against α_2 integrin or α_3 integrin and monoclonal antibodies against tubulin (Panel C). The chemiluminescence signal was quantified and normalized with respect to tubulin expression in an experiment that was representative of the four that were performed.

ter *snail* transcription had declined (Fig. 4B). Similarly, after in vitro wound healing, a transient induction of *snail* mRNA was observed, which probably corresponded to the transitional process in the cells next to the wound. Since the majority of the cells were not involved in the wound-healing process, no down-regulation of E-cadherin was observed in the total population (Fig. 4C).

UP-REGULATION OF α_2 INTEGRIN AND ACQUISITION OF A MIGRATORY PHENOTYPE

Failure of ultrafiltration in patients undergoing continuous ambulatory peritoneal dialysis is accompanied by peritoneal fibrosis. Therefore, we analyzed the characteristics of matrix-adhesion receptors in mesothelial preparations. A rapid up-regulation of α_2 integrin expression was already evident in cobblestone-like mesothelium derived from effluent (Fig. 5A and 5B). In contrast, expression of α_3 integrin was augmented in cobblestone-like cells and diminished in late stages of epithelial-to-mesenchymal transition (transitional and fibroblastic mesothelial preparations). Similarly, expression of the integrin-associated tetraspanins CD9 and CD151 was down-regulated as the transdifferentiation progressed. TGF-β1 plus interleukin-1β induced an increase in α_2 integrin in all the mesothelial preparations, whereas α_3 integrin was increased in omental samples and decreased in transdifferentiated transitional cells (Fig. 5C). Interleukin-1β potentiated the effects of TGF-β1, even though it did not affect integrin expression on its own.

Tetraspanins are functionally associated with cell migration.[30] The changes in the integrin reper-

toire and the switch from a keratin-based to a vimentin-based cytoskeleton could also affect the migratory capacity of mesothelial cells. We have observed that the transdifferentiation process was accompanied by a higher overall migratory capacity of mesothelial cells. Treatment with TGF-β1 plus interleukin-1β enhanced the haptotaxis to collagen, the main ligand for $\alpha_2\beta_1$ integrin. Migration toward laminin-5 followed the changes in the expression of its receptor, $\alpha_3\beta_1$ integrin; it was enhanced in epithelioid cultures and reduced in transitional and fibroblastic cells (data not shown).

EVIDENCE OF EPITHELIAL-TO-MESENCHYMAL TRANSITION OF MESOTHELIAL CELLS IN PERITONEAL TISSUE

Our data suggest that mesothelial cells undergo an epithelial-to-mesenchymal transition in the course of continuous ambulatory peritoneal dialysis. To confirm this hypothesis in vivo, we used immunohistochemical staining of peritoneal-biopsy specimens from nine patients who had been undergoing continuous ambulatory peritoneal dialysis for up to nine months and confirmed the loss of epithelial morphologic features on the monolayer of meso-

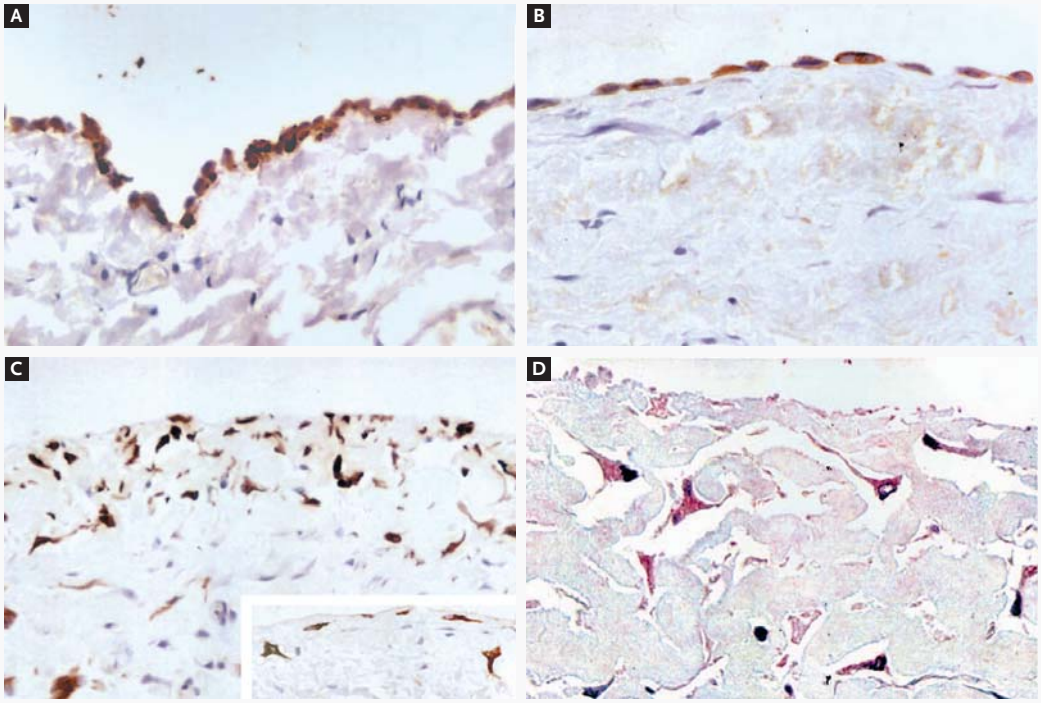

Figure 6. Evidence of Epithelial-to-Mesenchymal Transition of Mesothelial Cells in Peritoneal Tissue of Patients Undergoing Continuous Ambulatory Peritoneal Dialysis.

Images show immunohistochemical analysis of peritoneal-tissue samples stained with anticytokeratin antibodies (Panels A, B, and C, ×150) or with intercellular adhesion molecule 1 (ICAM-1) (Panel D, ×180), developed with peroxidase or fast red. Panel A represents control peritoneal tissue from a patient undergoing unrelated abdominal surgery (this sample is representative of eight tissue samples examined). In Panel B, a patient who had been undergoing dialysis for only six months already has a loss of mesothelial-cell polarity (this sample is representative of nine samples from patients who had been undergoing dialysis for nine months or less). Panel C (8 months of dialysis) and the inset (34 months of dialysis) represent the late stages of the transdifferentiation process in patients who have been undergoing dialysis for a long time; at this stage, fibroblast-like mesothelial cells are detected invading the fibrotic tissue (these samples are representative of eight samples obtained from patients who had been undergoing dialysis for 8 to 77 months). Panel D shows the staining with ICAM-1 of the biopsy specimen shown in Panel C.

thelial cells in the early stages of this type of dialysis (Fig. 6B). In biopsy specimens from eight patients who had undergone such dialysis for 8 to 77 months, the monolayer of mesothelial cells disappeared, and elongated mesothelial cells positive for cytokeratin and ICAM-1 were found embedded in the fibrotic tissue (Fig. 6C and 6D); these specimens corresponded to cultures of nonepithelioid mesothelial cells from effluent.

DISCUSSION

Peritoneal dialysis is an increasingly common alternative to hemodialysis. However, the procedure subjects mesothelial cells to high osmotic pressure and bioincompatible substances. Studies using standard histologic techniques on peritoneum from patients undergoing continuous ambulatory peritoneal dialysis show a complete loss of the monolayer of mesothelial cells and fibrosis, which might be responsible for the ultimate functional failure of the peritoneal membrane.[1] Our data show that mesothelial cells undergo a transition from an epithelial phenotype to a mesenchymal phenotype during peritoneal dialysis, with the induction of *snail* expression and a dramatic down-regulation of E-cadherin expression. Moreover, these findings are evidence of a direct and active role of mesothelial cells in the tissue fibrosis and failure of ultrafiltration in this process, generating new fibroblastic cells and leading to peritoneal fibrosis.

Previous studies have characterized the cobblestone-like mesothelial cells from peritoneal effluents as indistinguishable from mesothelial cells derived from omentum.[10] However, even early in continuous ambulatory peritoneal dialysis, a loss of cuboid morphology is observed both in vivo and ex vivo, accompanied by an induction of *snail* expression that down-regulates E-cadherin expression, even when cells retain an epithelioid appearance. If peritoneal dialysis is continued, long-term exposure to mechanical denudation, profibrotic factors such as TGF-β, and inflammatory cytokines may induce a complete transition of mesothelial cells, which could be responsible for tissue fibrosis and failure of ultrafiltration. Patients with recurrent episodes of peritonitis have high levels of expression of TGF-β[24] and have accelerated failure of ultrafiltration.[4]

The fact that mesothelial cells undergo epithelial-to-mesenchymal transition during continuous ambulatory peritoneal dialysis may change our view of the pathophysiology of ultrafiltration failure. Our data reveal a series of markers such as *snail*, E-cadherin, and α_2 integrin that are already modified in the early phases of the transdifferentiation process. In addition, ICAM-1 appears to be a potential marker that discriminates between mesothelial cells and fibroblasts. All these markers may be useful in the follow-up of patients undergoing peritoneal dialysis and in the development of new solutions for peritoneal dialysis. Furthermore, these data suggest new therapeutic targets that might ultimately prevent the fibrosis associated with continuous ambulatory peritoneal dialysis.

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6.1.2.2.- "Caracterización inmunohistoquímica de las subpoblaciones fibroblásticas en el tejido peritoneal normal y en la fibrosis inducida por la diálisis peritoneal"

Jiménez-Heffernan JA, Aguilera A, Aroeira LS, Lara-Pezzi E, Bajo MA, del Peso G, Ramírez M, Gamallo C, Sánchez-Tomero JA, Alvarez V, López-Cabrera M, Selgas R

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Caracterización inmunohistoquímica de las subpoblaciones fibroblásticas en el tejido peritoneal normal y en la fibrosis inducida por la diálisis peritoneal.

La fibrosis peritoneal es uno de los hallazgos morfológicos más frecuentemente encontrados en pacientes con DP. Las principales células implicadas en la fibrogénesis son los fibroblastos residentes y los fibroblastos derivados de la transición epitelio-mesenquimal de las células mesoteliales.

Objetivo: Establecer marcadores de daño peritoneal y los mecanismos patogénicos que expliquen la fibrosis peritoneal.

Métodos: Se realizó un estudio inmunohistoquímico de los fibroblastos peritoneales. Se analizaron muestras de peritoneo parietal en cuatro grupos de pacientes: controles sanos (n=15), pacientes urémicos sin DP (n= 17), pacientes urémicos tratados con DP (n= 27) y pacientes no urémicos con hernia inguinal (n=12). Para estudiar la conversión miofibroblástica de las células mesoteliales, se estudió la expresión de α-actina (α-SMA), desmina, citoqueratinas y cadherina E. También se analizó la expresión de CD34 en fibroblastos.

Resultados: Los fibroblastos de controles sanos y urémicos sin DP presentaron intensa expresión de CD34, pero no de marcadores miofibroblásticos ni mesoteliales. Los pacientes en DP mostraron un patrón inmunohistoquímico opuesto.

Conclusión: La expresión de citoqueratinas y cadherina E por las células fibroblásticas, y de α-SMA por las células mesoteliales y estromales, sugiere la presencia de transformación mesotelio-miofibroblástica en pacientes en DP. La

pérdida de expresión de CD34 se correlaciona con el grado de fibrosis peritoneal. El inmunofenotipo de los fibroblastos varía con la progresión de la fibrosis. Los miofibroblastos derivan tanto de la activación de fibroblastos residentes como de la conversión local de células mesoteliales.

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ORIGINAL ARTICLE

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Immunohistochemical characterization of fibroblast subpopulations in normal peritoneal tissue and in peritoneal dialysis-induced fibrosis

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Abstract Peritoneal fibrosis is one of the most common morphological changes observed in continuous ambulatory peritoneal dialysis (CAPD) patients. Both resident fibroblasts and new fibroblast-like cells derived from the mesothelium by epithelial-to-mesenchymal transition are the main cells involved fibrogenesis. In order to establish markers of peritoneal impairment and pathogenic clues to explain the fibrogenic process, we conducted an immunohistochemical study focused on peritoneal fibroblasts. Parietal peritoneal biopsies were collected from four patient groups: normal controls (n=15), non-CAPD uremic patients (n=17), uremic patients on CAPD (n=27) and non-renal patients with inguinal hernia (n=12). To study myofibroblastic conversion of mesothelial cells, α-smooth muscle actin (SMA), desmin, cytokeratins and E-cadherin were analyzed. The expression of CD34 by fibroblasts was also analyzed. Fibroblasts from controls and non-CAPD uremic patients showed expression of CD34, but no myofibroblastic or mesothelial markers. The opposite pattern was present during CAPD-related fibrosis. Expression of cytokeratins and E-cadherin by fibroblast-like cells and α-SMA by mesothelial and stromal cells supports that mesothelial-to-myofibroblast transition occurs during CAPD. Loss of CD34 expression correlated with the degree of peritoneal fibrosis. The immunophenotype of fibroblasts varies during the progression of fibrosis. Myofibroblasts seem to derive from both activation of resident fibroblasts and local conversion of mesothelial cells.

Keywords Peritoneal dialysis · Myofibroblasts · Fibrosis · Epithelial-to-mesenchymal transition

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Introduction

Continuous ambulatory peritoneal dialysis (CAPD) is an alternative to hemodialysis for the treatment of end-stage renal disease. Unfortunately, long-term exposure to hyperosmotic, hyperglycemic and acidic dialysis solutions often causes a low-grade chronic inflammation and injury to the peritoneum. Peritoneal fibrosis (or sclerosis) is one of the most common morphological changes observed in patients undergoing CAPD [9, 18, 29]. The degree of peritoneal fibrosis correlates with the time on dialysis and appears to be responsible for the progressive functional decline of the peritoneum, which ultimately may cause ultrafiltration failure. This functional decline of the

peritoneum may be accelerated by recurrent or severe episodes of peritonitis or hemoperitoneum [21]. The pathophysiological processes that lead to peritoneal impairment during long-term CAPD are not well understood. Peritoneal fibroblasts entrapped in the stroma have been classically considered as the main cells involved in peritoneal fibrosis. In contrast, the mesothelial cells have been considered, for long time, as mere victims of the tissue insults induced by CAPD treatments. We have previously demonstrated, in vivo and ex vivo, that mesothelial cells undergo a transition from epithelial to mesenchymal phenotype during CAPD, suggesting a direct and active role for mesothelial cells in the tissue fibrosis and ultrafiltration failure [34]. In addition, a recent report has demonstrated that mesothelial cells treated with transforming growth factor (TGF)-β acquire myofibroblastic properties in vitro, including the upregulation of α-smooth muscle actin (α-SMA) and collagen-I expression [32].

Myofibroblasts, which share biochemical and structural features with smooth muscle cells and fibroblasts, have been described in almost all human pathologies that course with wide-spread tissue fibrosis [19]. The origin of interstitial fibroblasts has been largely overlooked, and their lineage is not fully elucidated. One hypothesis argues that tissue fibroblasts derive from a subpopulation of circulating leukocytes, termed fibrocytes, which express the progenitor marker CD34 [1, 6]. In this context, subpopulations of CD34+ fibroblastic cells have been observed distributed throughout the connective tissue of many organs [25, 26]. Loss of expression of CD34 by tissue fibroblasts has been described in several pathological conditions associated with fibrosis [2, 3, 4, 7, 11, 14, 23]. However, the meaning of this loss of CD34 expression is still unknown and has been used mainly for diagnostic purposes. A second hypothesis propounds that interstitial fibroblasts are formed by local conversion from tissue epithelium by epithelial-to-mesenchymal transition (EMT), which is particularly evident during fibrotic repair following tissue injury [10]. In agreement with this hypothesis, we have demonstrated that peritoneal mesothelial cells undergo EMT in response to inflammatory and mechanical injuries induced by CAPD [34].

Regardless of the organ or tissue where fibrosis takes place, the cellular and molecular mechanisms that lead to fibrosis share many common features. In this study, we conducted immunohistochemical analyses focused on peritoneal fibroblasts to establish the pathogenic clues of the fibrogenic process during CAPD and to identify possible diagnostic markers of peritoneal impairment. More precisely, we evaluated immunohistochemical markers of myofibroblastic differentiation and EMT. In addition, we evaluated the loss of CD34 expression as marker of peritoneal fibrogenesis. In order to assess the specificity of these changes, we also analyzed peritoneal tissue samples with fibrosis unrelated to CAPD or uremia. For this purpose, pathological hernia sac specimens were obtained from patients with inguinal hernia.

Materials and methods

Patients

Biopsies were collected from four patient groups: (1) normal control samples (n=15) of parietal peritoneum obtained from autopsy cases and kidney donors; (2) uremic patients who had never undergone CAPD (n=17); (3) uremic patients on CAPD (n=27) and (4) non-renal patients with inguinal hernia (n=12). In renal patients, surgery was undertaken for renal transplantation, insertion or removal of the CAPD catheter or due to incidental abdominal conditions. Table 1 shows the most relevant clinical features of these patients, including supress episodes of peritonitis, time on CAPD and peritoneal function. Informed consent was obtained from all tissue donors.

Biopsy collection and processing

Except for the samples of visceral peritoneum and hernia sacs, all the remaining samples were obtained from the parietal peritoneum of the anterior abdominal wall. Samples measured 15–25×15–25 mm. In order to avoid mesothelial artifactual detachment, they were carefully manipulated and immediately fixed with neutral-buffered 3.7% formalin (pH 7.3) for 12–24 h. While immersed in formalin, they were gently attached to a flat surface to avoid retraction. Afterward, samples were cut and embedded in paraffin and then cut into 3-μm sections. When preparing the paraffin blocks, special efforts were made to orientate the samples perpendicular to the cutting surface. Sections were stained with hematoxylin-eosin, Masson trichromic and periodic acid-Schiff. For immunohistochemistry, paraffin sections were mounted on precoated slides, routinely deparaffinized and rehydrated and incubated with 3% hydrogen peroxide in methanol to block endogenous peroxidase activity. Antigen retrieval was performed using a citric acid solution (pH 6), which was heated with a microwave. Indirect immunohistochemical studies were performed by means of a dextran-polymer conjugate technique (EnVision+, Dako, Glostrup, Denmark). Table 2 shows the antibodies used in the study. For visualization, diaminobenzidine was used as chromogen. The sections were counterstained with a light hematoxylin stain.

Sample analysis

Histological interpretation was performed using recently published morphological criteria [29]. Morphological data regarding mesothelial status, thickness of submesothelial compact zone, hyalinizing vasculopathy and inflammation were recorded. In addition to the thickness of the submesothelial layer, the presence of dense, sclerotic areas of fibrosis was also evaluated. The density of the mesothelial cells was measured using a semiquantitative scale (grade 3, normal cell density; grade 0, complete denudation) as described by Plum et al. [18]. For this purpose, the whole mesothelial surface of the samples was analyzed. The mesothelial layer was highlighted using anti-cytokeratin antibodies (AE1/AE3). The thickness of the compact zone was measured with a graded (micrometer) ocular. We used the same method as to measure thickness of malignant melanoma. The pattern of α-SMA expression was depicted according to the number of positive fibroblast-like cells, distribution and clustering tendency. The number of positive cells was measured using a semiquantitative scale: (0) absence; (1) isolated positive cells (<15%); (2) frequent positive cells (15–35%) and (3) abundant positive cells (>35%). According to their tissue location, three areas were established: (a) superficial, when fibroblast-like positive cells were present in the mesothelial surface; (b) upper submesothelial level and (c) lower submesothelial area. Finally, the presence of clusters of α-SMA+ cells was recorded. Clusters were defined when a grouping tendency of positive cells was present. These groups were well defined, with high cellular density and showed a variable number of cells usually greater than 30. Clusters were surrounded by areas of α-SMA-

Table 1 Clinical data of the patients on peritoneal dialysis. *MTAC* mass transfer coefficent

Patient	Age (years)/sex	Time on peritoneal dialysis (months)	Episodes of peritonitis	Time since last peritonitis	Previous urea/Cr MTAC (ml/m)
1	55/female	77	6	14	14.2/3.6
2	44/female	8	0	-	16.9/5.5
3	61/female	21	0	-	21.9/7.4
4	71/femal	16	0	-	29/8.7
5	47/male	3	1	6	23.9/12
6	53/male	18	0	-	22/9.1
7	48/female	11	1	6	11.8/7.4
8	74/male	10	0	-	25.8/11.7
9	72/male	22	1	6	26.8/11.5
10	69/male	10	2		27.6/14.5
11	49/female	22	2	17	27.4/14
12	49/female	12	0	-	19/9.5
13	22/female	11	0	-	15.1/3.8
14	23/female	29	0	-	23/9
15	33/female	7	0	-	24.7/6.7
16	48/male	20	1	6	25.5/14
17	46/male	7	0	-	27.8/19.9
18	47/female	4	0	-	18.2/8.7
19	77/male	3	0	-	32.6/13
20	50/male	14	0	-	27.6/15.5
21	46/male	15	0	-	14.1/7.7
22	46/female	44	0	-	22/10.2
23	38/female	86	5	4	16.5/4.3
24	44/female	30	0	0	22.8/9
25	52/female	5	0	-	19.9/11.4
26	54/male	82	1	0.2	22.2/9.4
27	66/male	12	0	-	22.3/9.2

Table 2 Antibodies used in the study. *SMA* smooth muscle actin

Antigen	Clone	Source	Dilution
α-SMA	1A4	Dako	1/50
Muscle actin	HHF35	Dako	1/75
Desmin	D33	Dako	Prediluted
Cytokeratins	AE1/AE3	Immunon	Prediluted
E-cadherin	36	BD Biosciences	1/250
CD34	My10	Becton-Dickinson	1/30
CD34	QBEnd10	Dako	Prediluted
CD31	JC70A	Dako	1/20
Vimentin	V9	Dako	1/50

negative cells. The expression of cytokeratins and E-cadherin by submesothelial fibroblast-like cells was recorded as positive or negative with no further quantification. CD34 expression was measured using a semiquantitative scale: (0) absence; (1) scarce positive cells (<20%); (2) many positive cells with a homogeneous distribution and recognizable network; (3) abundant positive cells (>70%) forming a reticular network. Results of CD34 were compared with those of CD31 to avoid confusion with endothelial cells. To exclude hypocellularity as a reason for CD34 loss of expression, these results were compared with those of vimentin. Histological parameters from hernia sac specimens were not compared with the other specimens due to the different source of the sample, surgical manipulation and the impossibility for a correct orientation of the sample.

In toto experiment

For in toto studies, samples of normal parietal and visceral peritoneum (omentum) were taken from patients with elective abdominal surgical procedures. Patients had no infection, neoplasm or renal disorder. Samples were immediately introduced in a sterile recipient with saline solution. In sterile conditions, they were divided into four pieces, which were carefully extended in a corked petri-dish and fixed with pins. One specimen was fixed with formalin (basal control). The remaining three pieces were flooded with culture medium. One sample was stimulated with TGF-β (1/2000, 0.5 μl/ml), and IL-1 (1/1250, 1.2 μl/ml), during 24 h. A second one received the same stimulus during 48 h. The fourth sample remained in culture medium without treatment during 48 h (control). Samples were kept at 37°C and 5% CO_2. After 24 h or 48 h, the samples were washed with a buffered solution, fixed with formalin and processed routinely.

Statistical analysis

The mean and standard deviation of the semiquantitative scores were calculated for each group of patients. Data were evaluated using the SPSS 9.0 for Windows package. The differences in the scores among the groups were analyzed with the Kruskal-Wallis non-parametric test. Values of $P<0.05$ were considered significant. Differences between groups were ascertained by performing pair-wise comparison with the Mann-Whitney U test, and the level of significance was obtained with the adjusted Bonferroni method.

Results

Histological parameters in uremic non-CAPD and CAPD patients

The mesothelial layer was largely preserved in control and uremic non-CAPD patients (2.53±0.83 and 2.71± 0.47, respectively). As previously described [18, 29], CAPD patients showed a lower density of mesothelial cells (1.25±0.85, $P<0.05$). As shown in Table 3, normal controls and non-CAPD uremic patients showed similar thickness of the submesothelial compact zone (91.33± 46.65 and 111.76±36.91, respectively). In contrast, most

Table 3 Pathological and immunohistochemical findings in peritoneal biopsies. *MF* myofibroblast, *SMA* smooth muscle actin

Patients	Submesothelial thickness (μm)	MF	Superficial α-SMA+ cells	MF clusters	α-SMA+ mesothelial cells	CK+ fibroblasts	E-cadherin+ fibroblasts	CD34
Normal controls (n=15) mean±SD	91.33±46.65	-	-	-	-	-	-	2.47±0.52
Uremic controls (n=17) mean±SD	111.76±36.91	-	-	-	-	-	-	2.41±0.62
Peritoneal dialysis patients (n=27) mean±SD	277.92±155.16	70.4%	57.9%*	47.4%*	26.3%*	48.1%	25.9%	0.83±0.83
Hernia sac patients (n=12) mean±SD	Not present	91.7%	72.7%*	50%*	27.3%*	75%	33.3%	1±0.95

* Of the cases with myofibroblasts

patients on CAPD showed submesothelial fibrosis (277.92±155.16, P<0.05). The existence of CAPD patients with no fibrosis was responsible for wide variations within this group. Although non-CAPD uremic patients showed greater submesothelial thickness than normal controls, these differences were not statistically significant. Two patients on CAPD showed advanced lesions of hyalinizing vasculopathy (grades 3). Both were long-standing CAPD patients and showed submesothelial fibrosis. Another four showed grade 1–2 lesions. Except for two patients who showed a moderate chronic inflammatory infiltrate, no other histological signs of peritonitis were present.

Myofibroblasts are found in peritoneal fibrosis

Results are summarized in Table 3. Neither fibroblasts nor mesothelium from control or non-CAPD uremic patients showed α-SMA, muscle actin or desmin. Smooth muscle cells from the vessel wall were used as a positive internal control. Myofibroblasts were observed in the peritoneal samples of 19 (70.4%) patients on CAPD. In 9 (33.3%), they were a common finding (grades 2 or 3). They showed no relation with time on dialysis or intensity of fibrosis. Myofibroblasts were present in the three patients that were on CAPD treatment for less than 4 months. Of the 19 biopsies showing myofibroblasts, 11 (57.9%) were located exclusively in the upper submesothelial level, near the mesothelial surface (Fig. 1A, B). A remarkable finding was the clustering tendency of myofibroblasts. It was evident in nine cases (47.4%). Clusters were located in the upper submesothelial area and were surrounded by areas of α-SMA-fibroblasts (Fig. 1B). In many of these clusters, myofibroblasts coexisted with cytokeratin+ fibroblastic cells and loss of CD34 expression. Myofibroblasts showed no relation to blood vessels. In five cases (26.3%), there was evidence of α-SMA expression in mesothelial cells (Fig. 1D, E). The location, polygonal morphology and expression of cytokeratins permitted their recognition as mesothelial cells (Fig. 1F). In addition, 11 cases (57.9%) showed α-SMA+ fibroblast-like cells in the mesothelial surface (Fig. 1C). As described in a previous report [34], these cells expressed cytokeratins and corresponded to modified mesothelial cells.

Similar results were observed in the group of peritoneal samples from non-renal patients with inguinal hernia. Myofibroblasts were present in 11 of the 12 (91.7%) samples. In 7 (58.3%), they were a common finding. Of the 11 (72.7%) cases with myofibroblasts, 8 showed expression in superficial fibroblast-like cells. In 3 (27.3%), preserved mesothelial cells showed positive expression. A similar clustering tendency and superficial location of myofibroblasts was observed in this group.

Expression of cytokeratin and E-cadherin in a subset of submesothelial fibroblasts in patients with peritoneal fibrosis

Submesothelial fibroblasts from normal controls and non-CAPD uremic patients showed no expression of cytokeratins or E-cadherin. In both groups, the expression was confined to surface mesothelial cells. However, a subset of peritoneal fibroblasts from CAPD patients and hernia sac specimens showed cytokeratin and E-cadherin expression (Table 3). As in the case of α-SMA, cytokeratin expression was mainly located in the upper submesothelial area (Fig. 2A, B). Positive cells exhibited a spindle morphology and were completely surrounded by extracellular matrix (Fig. 2B, C). Some of these spindle cells also showed E-cadherin expression (Fig. 2D). This expression was located in the cytoplasm rather than in the cytoplasmic membrane, as it is normally seen in mesothelial cells. Both cytokeratin and E-cadherin expression are evidence of EMT of mesothelial cells during CAPD- and hernia-related peritoneal fibrosis. Given that the expression of cytokeratins and E-cadherin is gradually downregulated during the transdifferentiation of the mesothelial cells [34], it can be speculated that the fibroblast-like cells positive for these markers represent only a portion of the whole population of fibroblast that derive from the mesothelium.

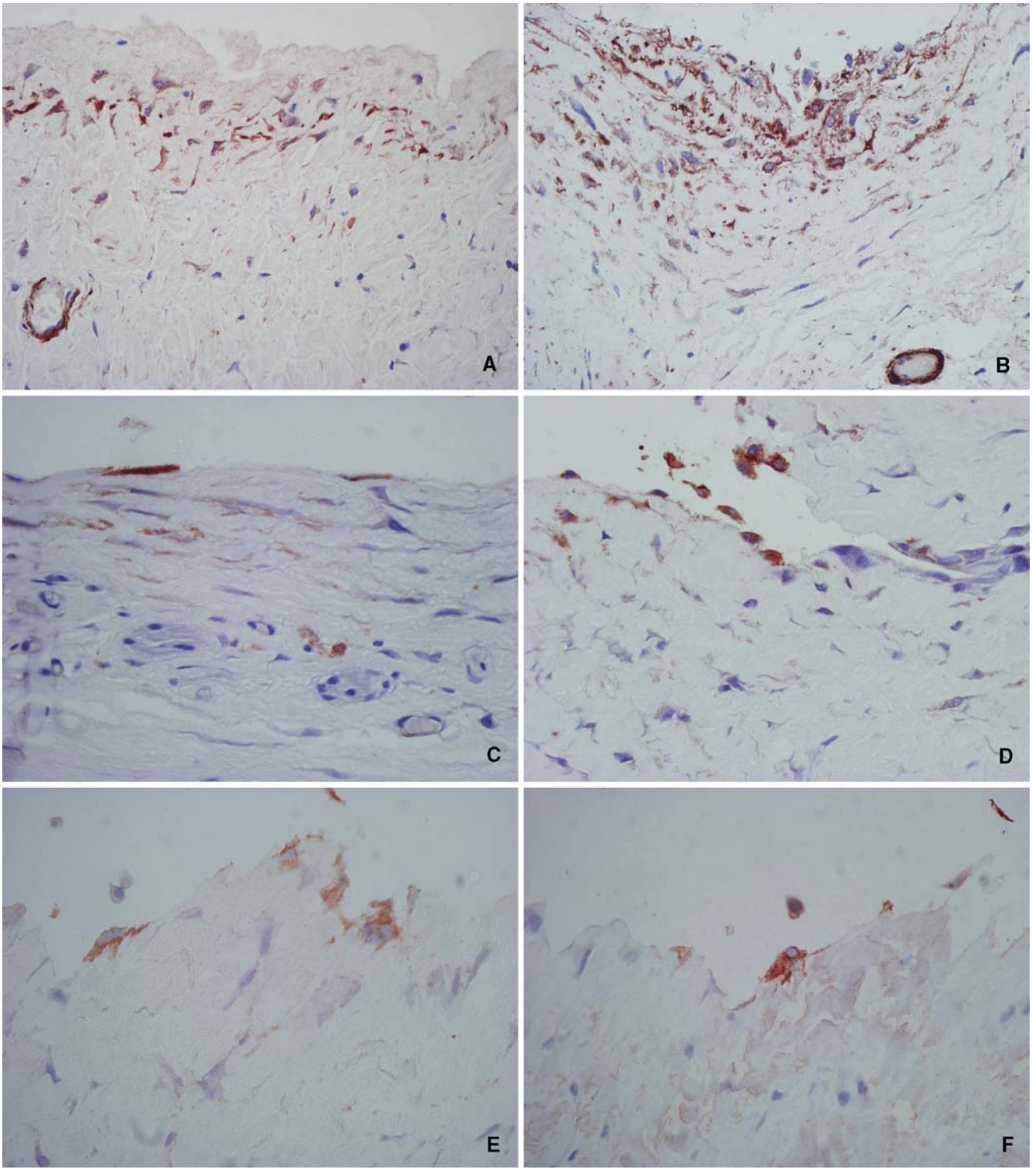

Fig. 1 Expression of α-smooth muscle actin in peritoneal samples from peritoneal dialysis patients. Myofibroblasts were located in the upper submesothelial level (**A**, **B**) and showed a clustering tendency (**B**). In addition to submesothelial cells, superficial cells with fibroblast morphology also showed positive expression (**C**). Immunoexpression was evident in a small subset of mesothelial cells with preserved morphology (**D**, **E**). Mesothelial cells also expressed cytokeratins (**F**, from the same patient as **E**)

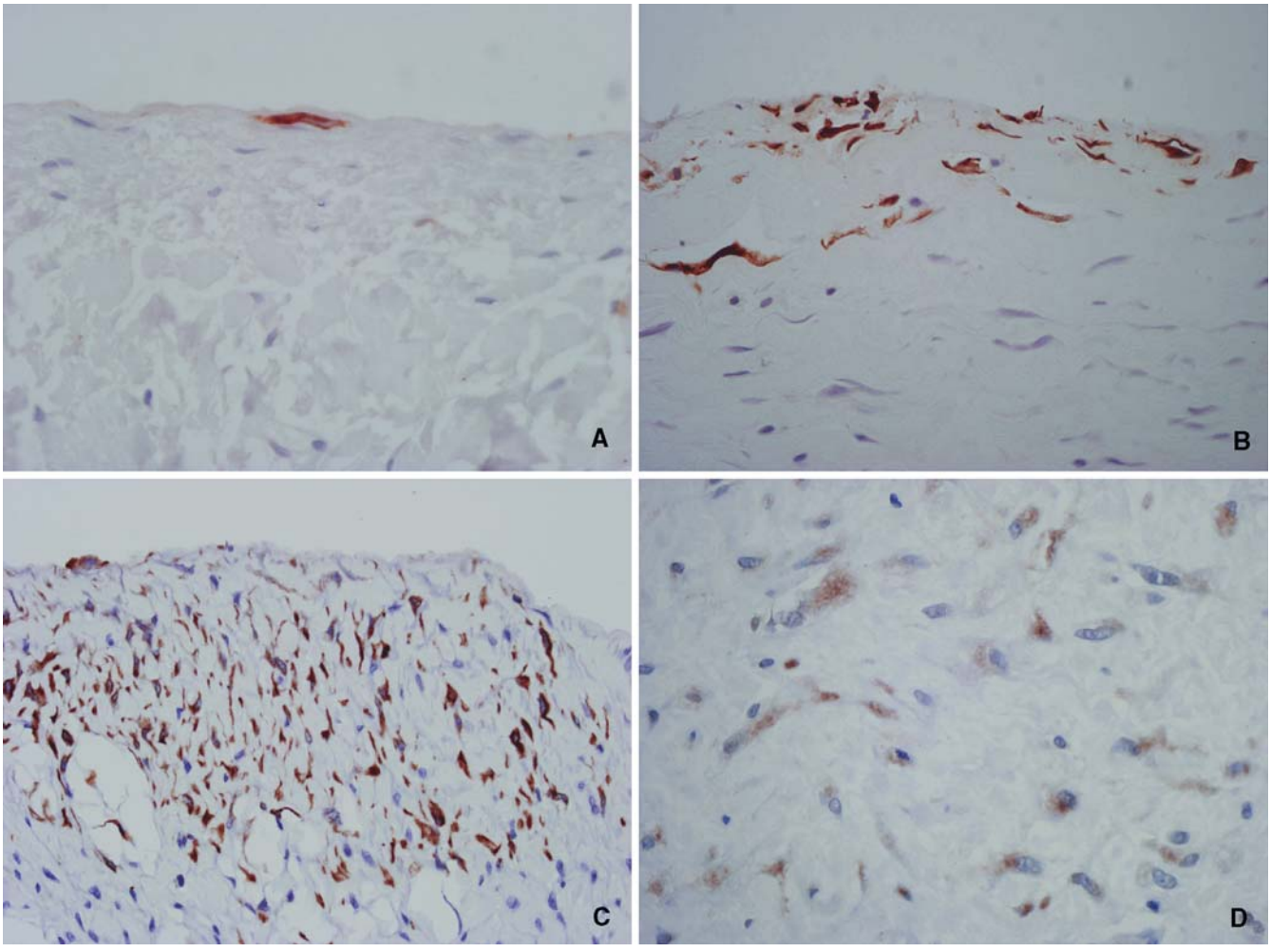

Fig. 2 Immunoexpression of cytokeratins was observed in superficial (**A**) and deeply located (**B**, **C**) cells with fibroblastic morphology. In addition, a subset of submesothelial cells showed cytoplasmic expression of E-cadherin (**D**)

The expression of CD34 on submesothelial resident fibroblasts is lost in patients with peritoneal fibrosis

Expression of CD34 was observed on resident fibroblasts of normal controls and non-CAPD uremic patients, with no differences between these two groups (2.47±0.52 and 2.41±0.62, respectively). The pattern of CD34 expression was a meshwork of slender cellular processes that extended from the immediate submesothelial layer to deeper levels (Fig. 3A, B). Immunoexpression was uniform, with no negative areas. Just below the mesothelial cell layer, the expression was greater, creating the impression of a continuous layer. The pattern of CD34 expression was similar in samples from visceral peritoneum. CD34 expression was evaluated using antibodies raised against different epitopes (My10, QBEnd/10). This suggests a true expression of the CD34 antigen rather than a cross-reaction phenomenon. The majority of patients on CAPD and peritoneal samples of hernia sac showed a partial loss of CD34 immunoexpression when compared with controls (0.83±0.82 and 1±0.95 respectively, $P<0.001$) (Fig. 3C, D, E). This was associated with areas of fibrosis and was greater in the upper submesothelial level (compact zone), with a tendency to show normal expression in the lower levels. Submesothelial thickness correlated inversely with CD34 immunoexpression ($r=-0.724$, $P<0{,}001$). In a few cases, the CD34 expression was variable, with negative and positive areas within the same sample (mixed pattern). They were graded according to the most abundant pattern of expression. Positive areas correlated with normal submesothelial morphology, with loose bands of collagen instead of sclerosis. The normal CD34 expression on endothelial cells was used as a positive internal control. Vimentin expression (Fig. 3F) of fibroblasts demonstrated that hypocellularity was not responsible for CD34 negative results.

TGF-β and IL-1 induce downregulation of CD34 in toto

To verify that the loss of CD34 correlated with resident fibroblasts activation, we performed an in toto experi-

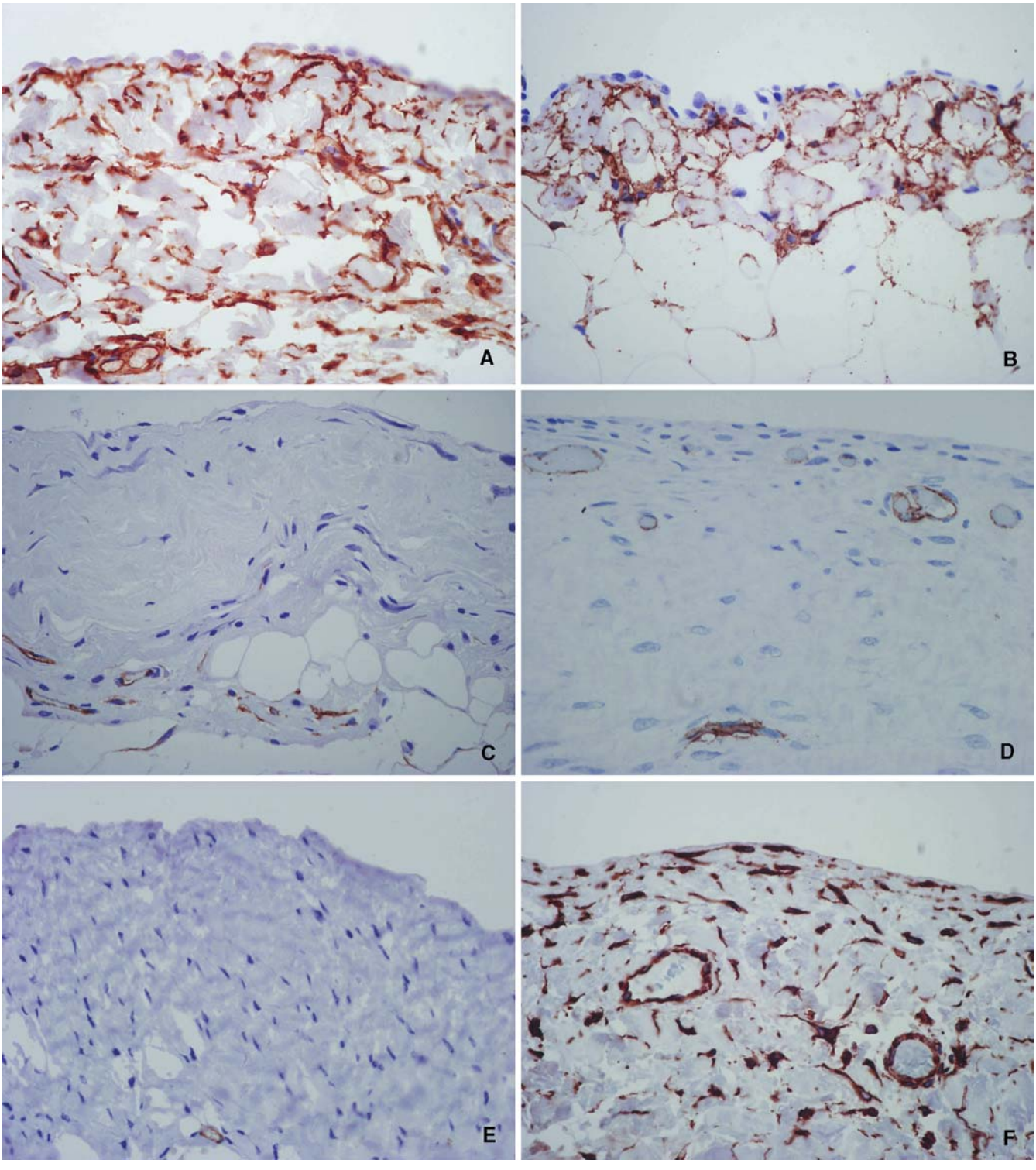

Fig. 3 Parietal (**A**) and visceral (**B**) peritoneum of normal controls showed an intense, submesothelial immunoexpression of CD34. Note the absence of expression in mesothelial cells. Samples with submesothelial fibrosis, either from peritoneal dialysis patients (**C**, **E**) or hernia sac specimens (**D**) showed a marked reduction in the expression of CD34. Its normal expression in endothelial cells (positive internal control) is preserved. The absence of expression is not due to cell loss since numerous negative fibroblasts are clearly visible and express vimentin (**F**)

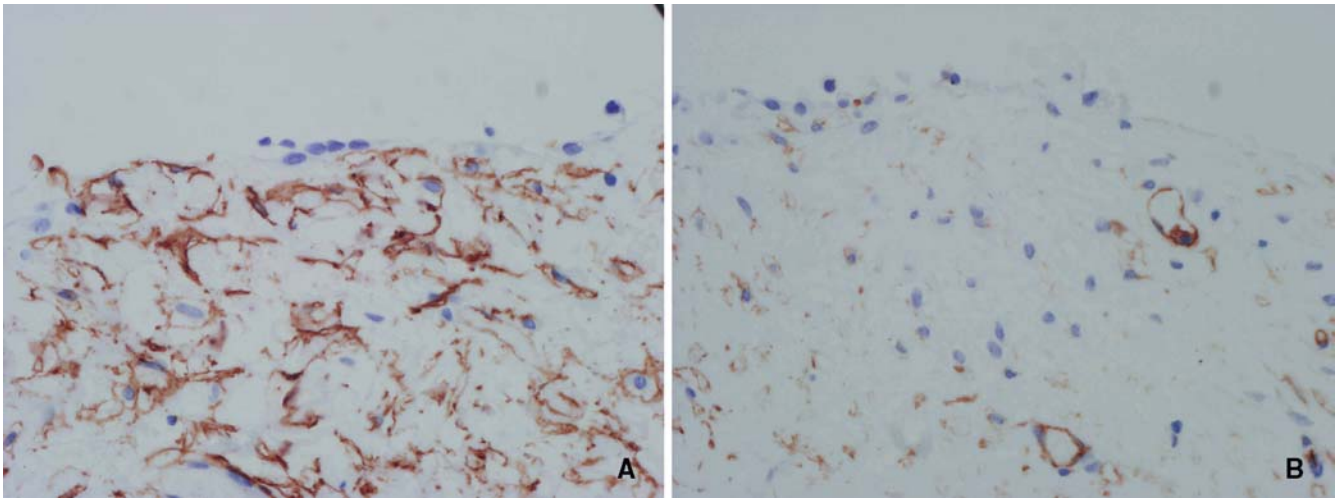

Fig. 4 In toto experiment. When compared with non-stimulated samples (**A**, control at 48 h), those that were treated with transforming growth factor-β and interleukin (IL)-1 for 48 h (**B**) showed a partial loss of CD34 expression

ment, in which samples of omental and parietal peritoneal tissue were split into four pieces, which were either treated or not with TGF-β and IL-1 for 24 h or 48 h. All the samples were processed simultaneously. The two control samples (at 0 h and 48 h, without TGF-β and IL-1) showed a preserved expression of CD34 in submesothelial fibroblasts (Fig. 4A). Similarly, samples incubated with TGF-β and IL-1 for 24 h showed a minimal downregulation of CD34 expression (not shown). However, a significant loss of expression was observed in the sample incubated with TGF-β and IL-1 for 48 h (Fig. 4B). These results indicate that activation of peritoneal fibroblasts induces the downregulation of CD34, as happens in endothelial cells [8].

Discussion

In this report, we show that the immunophenotype of submesothelial fibroblasts varies during the development of peritoneal fibrosis. The molecular markers analyzed show a sharp contrast between fibroblasts of normal and non-CAPD uremic peritoneal samples and those localized in peritoneal fibrotic tissue. Fibroblasts from normal controls and uremic patients show neither myofibroblastic nor mesothelial markers, but they exhibit an intense expression of CD34. The exact opposite immunophenotypical pattern is present under fibrogenic conditions. Our results indicate that myofibroblasts involved in fibrogenesis derive from both activation of resident fibroblasts and local conversion of mesothelial cells. In addition, this information is useful for the differentiation between quiescent and activated fibroblasts.

The simplest definition of myofibroblasts is that of smooth-muscle-like fibroblasts (α-SMA+ fibroblasts) [19, 20]. They show contractile capacity and are major producers of matrix molecules such as collagen, glycosaminoglycans, tenascin and fibronectin. According to the presence of vimentin (V), α-SMA (A) and desmin (D), different types of myofibroblasts have been described. Those identified in our study corresponded to VA-type myofibroblasts. Myofibroblasts have been implicated in almost all the fibrogenic situations that take place in human pathology [19]. Only few reports have mentioned their presence in CAPD patients [13, 15, 22]. The present study demonstrates that myofibroblasts are a common finding during peritoneal fibrosis, but their presence and quantity are not related to time on dialysis, nor to the degree of fibrosis. Similar results were obtained by Mateijsen et al. [13] and Shioshita et al. [22]. Our series included three patients with less than 4 months on dialysis who showed myofibroblasts. These findings demonstrate that myofibroblastic differentiation takes place during the initial phase of treatment and that it precedes the morphological appearance of fibrosis. The constant and periodic exposure to dialysis fluids will maintain the stimuli for myofibroblastic activation, independently of the time on dialysis or fibrosis status. Another remarkable finding of this study was the tendency of myofibroblasts to distribute in clusters, suggesting the existence of a local transforming environment.

Regarding the origin of myofibroblasts, transition from epithelial cells has been described in renal tubular cells under profibrogenic conditions [10, 24, 33]. A recent in vitro report describes a similar conversion of mesothelial cells into myofibroblasts [32]. Our study of the location and distribution of α-SMA+ cells supports such a conversion in vivo. Mesothelial cells and modified mesothelial cells expressing α-SMA were observed. In addition, a gradient in the distribution of myofibroblasts was evident. Most were located in the upper submesothelium, in continuity with the mesothelial surface. Myofibroblastic conversion of mesothelial cells must be regarded as another proof of EMT. Other useful markers that prove such a transition are cytokeratins and E-cadherin. Under normal conditions, both molecules are present in meso-

thelial cells and absent in fibroblasts. This study has shown that, during fibrosis, these markers are expressed in a subset of fibroblast-like cells. The fact that EMT can be induced by advanced glycation end products (AGEs) [17] is very relevant information, since AGEs are thought to play an important pathogenic role in CAPD.

In this study, we have also demonstrated an intense immunoexpression of CD34 in normal submesothelial fibroblasts. Although mentioned in regard to the pleura [5, 28], it has not been evaluated previously in the peritoneum. The pattern is similar to that observed in other tissues [16, 27, 30, 31] and consists of a meshwork of cellular prolongations that extend homogeneously from the immediate submesothelial layer to deeper levels. This pattern is preserved in non-CAPD uremic patients. However, it diminishes and disappears during peritoneal fibrosis. A similar phenomenon of CD34 loss of expression has been described in other types of fibrosis, such as that seen in the skin [2, 3, 11, 23] and in the fibrotic response associated with several types of carcinoma [4, 7, 14]. The role of CD34+ fibroblastic stromal cells in the fibrotic process is not well understood, and the exact meaning of CD34 loss of expression is unknown. As it happened in our study, loss of CD34 expression occurs in cell cultures of endothelial cells. It can be induced by inflammatory mediators, such as interleukin-1β, interferon-γ or tumor necrosis factor-α [8, 12]. These molecules downregulate CD34 and induce upregulation of the adhesion molecule ICAM-1 [12]. A similar phenomenon seems to occur in submesothelial fibroblasts during tissue fibrosis. In addition to CD34 downregulation, we also have observed ICAM-1 expression in submesothelial fibroblasts from CAPD patients [34].

Although, in our samples, the appearance of myofibroblasts was associated with loss of CD34 expression, it is difficult to assess if the two are related. In a few cases, myofibroblasts coexisted with a normal CD34 pattern. However, in the majority of cases, and mainly in those with clusters, an inverse relation was noted. A similar phenomenon of CD34 loss and myofibroblastic transformation has been described for tissue and circulating CD34 fibrocytes [1, 7]. It has been observed under fibrogenic conditions and can be induced by TGF-β [1].

One of the conclusions from this study is that, in contrast to CAPD patients, fibroblasts from non-CAPD uremic patients (predialysis or hemodialysis) showed a normal immunophenotypical pattern. The fact that these patients showed no significant submesothelial thickness when compared with normal controls correlated with the immunohistochemical findings. Another important conclusion is that, except for hyalinizing vasculopathy, the findings described are not specific to CAPD. Similar markers and mechanisms have been observed in fibrotic peritoneal samples (hernia sacs) obtained from non-renal patients with inguinal hernia. It reflects that the mechanisms leading to fibrosis are similar, regardless of the causal agent (chemical or mechanic).

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6.1.2.3.- “Diferenciación miofibroblástica en la esclerosis peritoneal simple”

del Peso G, Jiménez-Heffernan JA, Bajo MA, Hevia C, Aguilera A, Castro MJ, Sánchez-Tomero JA, López-Cabrera M, Selgas R.

Int J Artif Organs 2005; 28(2): 135-140

Este trabajo responde a los objetivos 1 y 3

Diferenciación miofibroblástica en la esclerosis peritoneal simple

La esclerosis (o fibrosis) peritoneal simple es una de las lesiones morfológicas más frecuentes de los pacientes tratados con DP. Los fibroblastos submesoteliales son la fuente principal de matriz extracelular en la membrana peritoneal. En situaciones de fibrosis, los fibroblastos expresan α-actina (α-SMA) y se transforman en miofibroblastos. En pacientes con largas estancias en DP o con problemas funcionales peritoneales, se ha descrito la presencia de miofibroblastos a nivel peritoneal.

Objetivos: Analizar la presencia de miofibroblastos en peritoneo de pacientes en DP con esclerosis peritoneal simple y en urémicos no tratados con DP. Estudiar la correlación entre la presencia a nivel peritoneal de miofibroblastos y otros parámetros morfológicos y funcionales.

Métodos: Se analizaron biopsias en tres grupos de pacientes: controles sanos (n=15), pacientes urémicos no tratados con DP (n=16) y pacientes urémicos en tratamiento con DP (n=32). En todos los casos, se estudiaron parámetros morfológicos y funcionales peritoneales, así como la expresión inmunohistoquímica de α-SMA. En un subgrupo de pacientes, se analizó la expresión de VEGF, proteína anti-apoptótica bcl-2 y receptor de progesterona.

Resultados: En un 56.3% de pacientes en DP con esclerosis peritoneal simple se observaron miofibroblastos peritoneales, la mayoría distribuidos en el área submesotelial superficial. Ninguna de las biopsias de los controles sanos ni de pacientes urémicos sin DP presentó miofibroblastos. En el grupo de pacientes en DP, la presencia de miofibroblastos no se asoció con el tiempo en diálisis, MTC de urea, MTC de creatinina, episodios de peritonitis, grosor submesotelial,

vasculopatía hialinizante o preservación de la capa mesotelial. En un subgrupo de pacientes en DP, se evidenció expresión de VEGF en fibroblastos submesoteliales, pero no expresión de bcl-2 o de receptor de progesterona. Conclusiones: Los miofibroblastos son marcadores sencillos y fiables de fibrosis, dado que aparecen en etapas iniciales del tratamiento con DP, incluso en pacientes con pocas alteraciones estructurales. No son exclusivos de pacientes con peritonitis esclerosante, fallo de ultrafiltración o largas estancias en DP. Su ausencia en pacientes urémicos sin DP sugiere que en la fibrosis relacionada con la uremia, los miofibroblastos no tienen una participación significativa.

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Peritoneal Sclerosis

Myofibroblastic differentiation in simple peritoneal sclerosis

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ABSTRACT: Objective: *To analyze the presence of myofibroblasts in a series of peritoneal dialysis (PD) patients with simple sclerosis and non-PD, uremic patients. Since there is a close correlation between active fibrosis and myofibroblastic differentiation we wanted to test if myofibroblasts are present in uremic, non-PD peritoneal samples. To determine if there are correlations between myofibroblastic presence and other functional and morphologic peritoneal parameters.*
Methods: *Biopsies were collected from three patient groups: 1) Normal control samples (n=15) of parietal and visceral peritoneum 2) non-PD uremic patients (n=16); and 3) uremic patients on PD (n=32). Peritoneal morphologic and functional parameters and immunohistochemical expression of α-smooth muscle actin was analyzed in each case. Vascular endothelial growth factor (VEGF), bcl-2 anti-apoptotic protein, and progesterone receptor was evaluated in a subset of cases.*
Results: *Myofibroblasts were present in 56.3% of the patients with PD-related simple sclerosis. In most cases they were distributed in the upper submesothelial area. None of the biopsies from normal controls and uremic, non-PD patients showed myofibroblasts. Within the group of PD patients, myofibroblasts showed no correlation with time on dialysis, urea/creatinine MTAC, episodes of peritonitis, submesothelial thickening, hyalinizing vasculopathy or mesothelial status. In a subset of PD patients VEGF expression was observed in submesothelial fibroblastic cells. No expression of progesterone receptor or bcl-2 was observed.*
Conclusions: *Myofibroblasts are a reliable and simple indicator of fibrosis since they appear in early stages of PD treatment and in patients with minor morphologic anomalies. They are not exclusive of patients with sclerosing peritonitis, ultrafiltration loss or long standing treatment. Their absence in non-PD, uremic patients suggest that uremia-related fibrosis takes place without a significant participation of myofibroblasts. (Int J Artif Organs 2005; 28: 135-40)*

KEY WORDS: Fibrosis, Myofibroblast, Peritoneal dialysis, Uremia

INTRODUCTION

Simple peritoneal fibrosis (or sclerosis) is one of the most common morphologic changes observed in patients undergoing peritoneal dialysis (PD) treatment (1, 2). In the peritoneal membrane the extracellular matrix synthesis is performed chiefly by fibroblastic submesothelial cells. Under situations of fibrosis many of these fibroblastic cells express α-smooth muscle actin (SMA), a feature characteristic of myofibroblastic differentiation (3, 4). The appearance of myofibroblasts at sites of wound healing and tissue repair/fibrosis is associated with active fibrosis and wound contraction. The myofibroblast is a mesenchymal cell with intermediate features between a

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fibroblast and a smooth muscle cell, characterized by the expression of α-smooth muscle actin isoform (3, 4). They have been described in almost all the fibrogenic conditions that take place in human pathology, including PD-related fibrosis (5-10). Previous reports describing myofibroblasts in the peritoneum of PD patients have been performed mostly on biopsies from patients with sclerosing peritonitis, ultrafiltration loss or long-standing PD treatment. In the present series the majority of patients showed simple fibrosis and an adequate peritoneal function, allowing an analysis of patients under "normal" PD conditions. Different aspects of PD treatment, including fluid bioincompatibility, and peritonitis episodes are pro-fibrogenic. In addition, uremia itself is considered a fibrogenic condition. Several reports describe morphologic and functional abnormalities of the uremic peritoneum not related to PD treatment (2, 11, 12). One of the most relevant changes is fibrosis and submesothelial thickening. Since there is a close correlation between active fibrosis and myofibroblastic differentiation we wanted to test if myofibroblasts are present in the peritoneum of uremic, non-PD subjects. In order to clarify the role of myofibroblasts in uremia and PD related simple sclerosis we have studied their presence in a series of peritoneal biopsies using immunohistochemistry. In PD patients a correlation study with functional and morphologic features was done.

PATIENTS AND METHODS

Patients

Biopsies were collected from three patient groups: 1) normal control samples (n=15) of parietal peritoneum (n=9) and visceral peritoneum (n=6); 2) uremic patients who had never undergone PD (n=16); and 3) uremic patients on PD (n=32). In PD patients surgery had been done for renal transplantation (n=15), inguinal hernia sac (n=9), nephrectomy (n=3), incidental abdominal conditions (n=2), and insertion of the PD catheter (n=3). Except for the samples of visceral peritoneum all the remaining were obtained from the parietal peritoneum from the anterior abdominal wall. Hernia sac specimens were not included in the study since they show pathologic features unrelated to PD (due to mechanical trauma and inflammation). Seventeen of the PD patients were women and 15 men, with a mean age of 49±16 years (range from 20 to 80). Nine patients were on continuous ambulatory PD and 23 on automatized PD. Mean time on dialysis was 18.2 months (range from 1.9 to 79.4). Peritoneal functional parameters were derived from the last peritoneal equilibration test preceding the biopsy performance. Mass transfer coefficient (MTAC) of urea and creatinine were recorded in each case. In three patients two biopsies obtained at different moments were available. In two of them the first one preceded PD treatment while the second was performed after 3 and 12 months on PD. In the remaining patient, the first one was obtained during PD treatment and the second after PD withdrawal and six months on hemodialysis.

Tissue processing and pathologic analysis

Tissue samples measured 1.5-2x1.5-2 cm. They were carefully manipulated and immediately fixed with neutral-buffered 3.7% formalin (pH 7.3) for 12-24 hours. While immersed in formalin they were attached to a flat surface to avoid retraction. Samples were processed as previously described (13). They were paraffin-embedded and stained with hematoxylin-eosin. Indirect immunohistochemical studies were performed by means of a dextran-polymer conjugate technique (EnVision+, Dakocytomation). Antigen retrieval was performed using a citric acid solution (pH 6) which was heated with a microwave. The following primary antibodies were tested: α-smooth muscle actin (SMA), muscle actin, CD31, bcl-2 (all from Dakocytomation), vascular endothelial growth factor (VEGF) (Zymed), estrogen and progesterone receptors (Novocastra), and CD34 (Becton-Dickinson). Morphologic analysis was focused on mesothelial status, fibrosis (thickness of compact zone), hyalinizing vasculopathy and inflammation. The thickness of the submesothelial compact zone was measured by a graded eyepiece. A semiquantitative score of submesothelial thickness was given: (0) minor or no relevant thickening (<100 μm); (1) moderate fibrosis (greater than 100 but lower than 300 μm); and (2) prominent fibrosis (>300 μm). α-SMA immunohistochemical results were expressed according to the number of positive fibroblast-like cells and distribution. The number of positive cells was measured using a semiquantitative scale: (0) absence; (1) isolated positive cells (<10%); (2) frequent positive cells (10-30%); and (3) abundant positive cells (>30%). To avoid confusion with vascular smooth muscle cells, α-SMA results were compared to those obtained with CD31 (endothelial cell

marker). Following on the recent description of Bongiovanni et al (14) we evaluated estrogen and progesterone receptors and bcl-2 in 20 cases (7 controls, 7 uremic, non-PD and 6 PD patients). VEGF expression was analyzed in 5 controls and 8 PD patients with fibrosis and myofibroblasts. Results were recorded as positive or negative with no quantification.

Statistical analysis

The mean and standard deviation of the semi-quantitative scores were calculated for each group of patients. Data were evaluated by using the SPSS 9.0 for windows package. The differences in the scores among the groups were analyzed with the Kruskal-Wallis non-parametric test. Differences between groups were ascertained by performing pairwase comparison with the Mann-Whitney U test. Values of $p<0.05$ were considered significant.

RESULTS

Normal controls and uremic non-PD patients showed similar thickness of the submesothelium (0.44±0.3 and 0.56±0.3, respectively). Peritoneal biopsies from PD patients showed greater submesothelial thickness (1.66± 0.4, $p<0.05$). None of the biopsies from normal controls including samples of visceral peritoneum showed α-SMA, or muscle actin expression in submesothelial fibroblasts. Similarly, no myofibroblasts were observed in the samples of uremic patients not receiving PD treatment. In all cases smooth muscle cells from the blood vessels were used as a positive control (Fig. 1A). As previously reported fibroblasts did express CD34 (13) (Fig. 1B). Myofibroblasts were present in 18 of the 32 (56.3%) patients undergoing PD treatment. In 11 it was a common finding (grades 2 or 3). Myofibroblasts distributed mainly in the upper submesothelial area (Fig. 1C). In many cases they were present in the peritoneal surface (Fig. 1D). Within the group of PD patients, myofibroblasts showed no correlation with time on dialysis, urea/creatinine MTAC, episodes of peritonitis, submesothelial thickening, hyalinizing vasculopathy or mesothelial status. Three patients had two different biopsies. In two patients in whom the first one preceded PD treatment myofibroblasts were exclusively seen in the second one (while on PD). In the third patient, the first biopsy (on PD) showed myofibroblasts while the second one, six months after PD withdrawal and hemodialysis treatment, was devoid of them. Estrogen and progesterone receptors and bcl-2 protein were evaluated immunohistochemically with negative results. None of the 20 cases analyzed showed expression on fibroblasts, endothelium or mesothelium. In the five normal controls evaluated VEGF expression was limited to mesothelial and endothelial cells (Fig. 1E). Three of the eight PD patients analyzed also showed positive expression in a subset of fibroblastic submesothelial cells (Fig. 1F)

DISCUSSION

In this report we have shown that myofibroblasts are a common finding in PD patients. 56.3% of the biopsies showed myofibroblasts and in 34.4% they were a frequent feature. On the contrary, the peritoneum of normal controls and uremic patients showed no myofibroblasts. The biopsies analyzed were obtained from PD patients with simple fibrosis and most of them had an adequate peritoneal function. Myofibroblasts were present in many biopsies showing minor changes, revealing that myofibroblastic activation is an early phenomenon not exclusively of patients with sclerosing peritonitis, ultrafiltration loss or long standing treatment. In a previous report we described myofibroblasts in biopsies from patients with less than four months on PD treatment, and no relevant complications or fibrosis (13). In the present series myofibroblasts showed no relation to time on dialysis, submesothelial thickness, or peritoneal function. However, we believe that if patients with simple fibrosis are compared to those with sclerosing peritonitis these will show more intense and extensive evidence of myofibroblastic differentiation.

Myofibroblasts were first described as an important cellular component of wound repair (15). In addition to their protective role in wound healing they have been implicated in a variety of pathologic conditions involving fibrosis and tissue remodelling. They produce extracellular matrix molecules and have contractile capacity, a peculiarity that probably participates in the progressive visceral encapsulation seen in patients with sclerosing peritonitis. Another interesting feature of the myofibroblastic/fibroblastic peritoneal cell population is their capacity to produce VEGF. In addition to mesothelial and endothelial cells we have observed VEGF expression

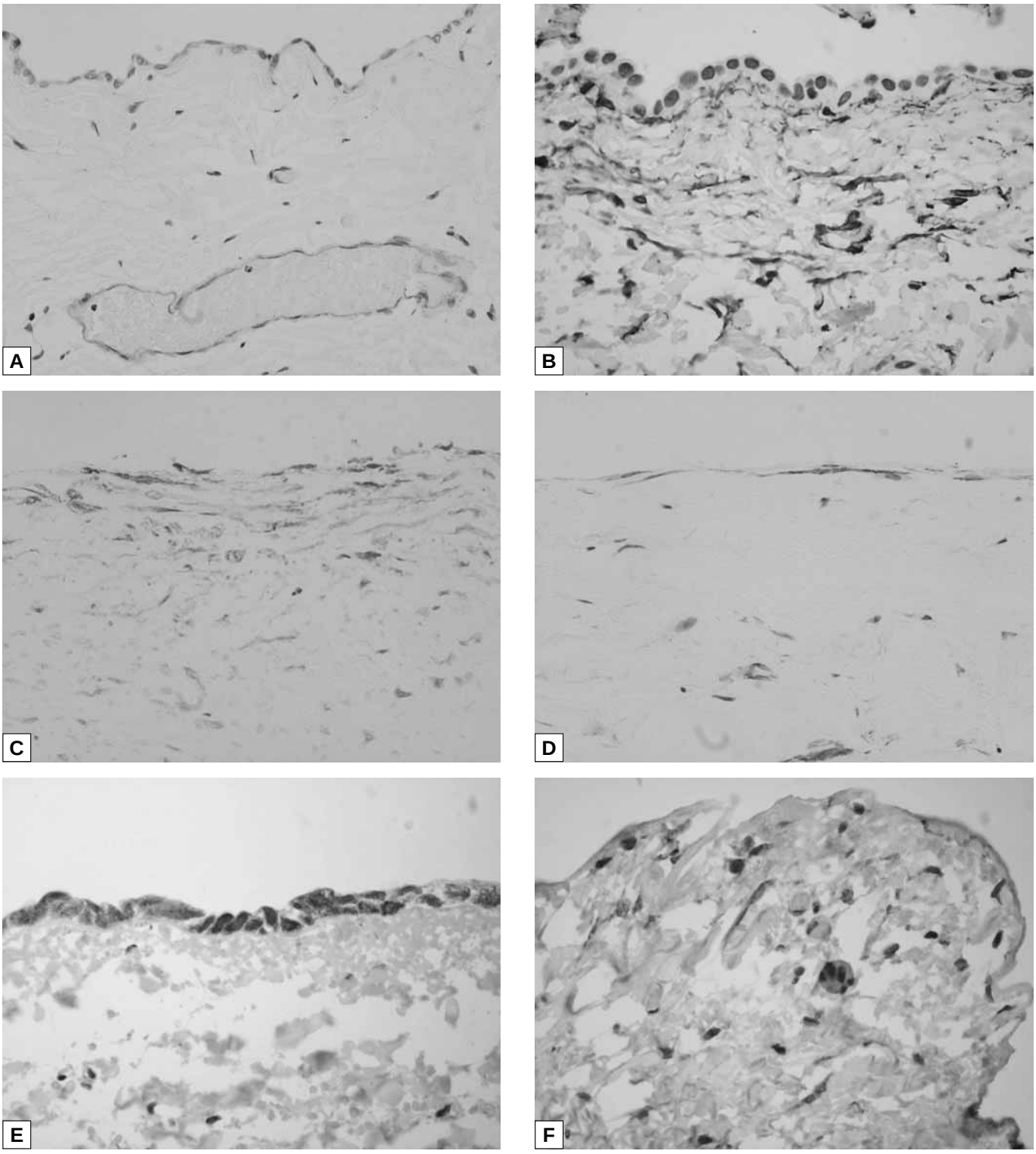

Fig. 1 - *The normal peritoneum shows α-smooth muscle actin expression limited to blood vessels;* **(A)** *peroxidase x100 and numerous CD34+ fibroblasts;* **(B)**, *peroxidase x104. Myofibroblasts in a case of peritoneal dialysis-related simple sclerosis showing a superficial distribution;* **(C)** *peroxidase x100. In some cases myofibroblasts are identified in the peritoneal surface;* **(D)** *peroxidase x125. The mesothelial layer from a normal control showing expression of vascular endothelial growth factor (VEGF);* **(E)** *peroxidase x150. Expression of VEGF in submesothelial fibroblastic cells, in a patient with simple sclerosis. Note the absence of mesothelial layer,* **(F)** *peroxidase x150.*

in fibroblastic cells of PD patients. Similar results have been obtained in animal models (16, 17). In other situations of peritoneal fibrosis (hernia sacs), not related to PD, we have also observed VEGF expression in fibroblastic cells (unpublished data). VEGF production would contribute to neoangiogenesis and ultrafiltration failure. In a recent report Bongiovanni et al describe partial expression of progesterone receptor in fibroblasts from pleural samples with chronic pleuritis (14). This study was motivated by the observation of progesterone receptor and bcl-2 expression in solitary fibrous tumor, a neoplasm thought to derive from submesothelial fibroblasts. Our samples showed no expression of progesterone receptor or bcl-2 anti-apoptotic protein. As stated by Bongiovanni et al the presence of inflammatory changes in pleural specimens may stimulate steroid hormone receptors, justifying their expression. In normal wound-healing and in self-limiting models of fibrosis, myofibroblasts gradually disappear (through apoptosis) as the active fibrotic phase is finished (3). At least in one of our patients we could demonstrate a similar reversibility of this process. In pathologic situations such as pulmonary fibrosis, renal sclerosis and liver cirrhosis, myofibroblastic activation and fibrosis persist despite the suppression of the initial causative agent (3). In PD treatment, encapsulating sclerosing peritonitis represents this situation of autonomous myofibroblastic activation and disease progression. During the last few years several studies have clarified the origin of myofibroblasts. Three different sources are admitted. The first one is transdifferentiation from resident fibroblasts or smooth muscle cells (3). A second source is transition from nearby epithelial cells, a phenomenon known as epithelial to mesenchymal transition, and proved to occur in the peritoneum (18, 19). This transition occurs in various steps that involve loss of epithelial cell adhesion, *de novo* α-SMA expression, disruption of the basement membrane and cell migration (20,21). Finally, myofibroblasts may be derived from the bone marrow and non-resident, circulating fibrocytes (22-25). This third source has not yet been demonstrated in the peritoneum. In our series, myofibroblasts showed a tendency to locate in the upper submesothelial area, even at the surface. This distribution supports a partial mesothelial origin. No clear relation was seen with blood vessels.

In contrast to PD patients, uremic patients not undergoing PD treatment showed no signs of myofibroblastic differentiation. In our series we found no significant morphologic differences between normal and uremic peritoneal samples. Neither submesothelial thickness nor qualitative aspects of the stroma differed considerably. A similar absence of myofibroblasts in non-PD, uremic peritoneum is described in other studies (6, 7, 12). This data suggest that uremia-related fibrosis takes place without a significant participation of myofibroblasts. In conclusion, myofibroblasts are a reliable and simple indicator of fibrosis since they appear in early stages of PD treatment and in patients with minor morphologic anomalies. They seem to participate in many of the pathologic conditions related to PD treatment (fibrosis, angiogenesis) but not in uremia-related peritoneal changes.

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6.1.2.4.- “Distribución tisular de las lesiones de vasculopatía hialinizante en pacientes en diálisis peritoneal. Un estudio autópsico”

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Este trabajo responde al objetivo 1

Distribución tisular de las lesiones de vasculopatía hialinizante en pacientes en diálisis peritoneal. Un estudio autópsico.

Se considera que la uremia puede inducir alteraciones estructurales de la membrana peritoneal, como la vasculopatía hialinizante (VH). La uremia es una enfermedad sistémica; por lo que es esperable que, si es capaz de inducir VH, ésta pueda ser detectada no solo en peritoneo, sino también en tejidos extraperitoneales.

Objetivo: Con el fin de valorar la contribución de la uremia a la severidad de las lesiones de VH, se realizó un estudio de autopsias de pacientes en DP con lesiones peritoneales severas de VH.

Métodos: Se estudiaron muestras de tejido procedentes de siete autopsias de pacientes con largas estancias en DP con lesiones severas de VH a nivel peritoneal. Se revisaron las preparaciones histológicas de peritoneo, todos los órganos abdominales, corazón, pericardio, pulmones, pleura visceral y sistema nervioso central.

Resultados: Las lesiones a nivel peritoneal fueron severas en todos los pacientes, con intensa VH, fibrosis y presencia variable de inflamación, fibrina y calcificación. Las lesiones de VH se limitaron al peritoneo, salvo en un paciente diabético, que presentó lesiones focales de VH en la submucosa intestinal. El resto de tejidos extraperitoneales no mostraron lesiones de VH.

Conclusiones: Los pacientes en DP con intensa VH a nivel peritoneal no muestran lesiones significativas de VH en los vasos extraperitoneales. Esto sugiere que la severidad de la vasculopatía está principalmente relacionada con factores asociados a la DP. La uremia puede ser un factor que contribuya

al desarrollo de VH, pero no parece responsable de la severidad de la lesión.

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ORIGINAL ARTICLE

Tissue distribution of hyalinazing vasculopathy lesions in peritoneal dialysis patients
An autopsy study ☆

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Abstract

Uremia is considered capable of inducing structural anomalies of the peritoneum, including hyalinizing vasculopathy (HV). To further elucidate the contribution of uremia to the severity of HV, we performed an autopsy study of peritoneal dialysis (PD) patients with severe peritoneal HV lesions. Uremia is a systemic condition and, if capable of inducing HV, it will be expected to be detected outside the peritoneum. Seven autopsy cases of PD patients showing prominent peritoneal HV lesions were selected. Histological slides from the peritoneum, abdominal organs, heart and pericardium, lungs, visceral pleura, and central nervous system were reviewed. Peritoneal lesions were intense in all patients with prominent HV, fibrosis, and a variable presence of inflammation, fibrin, and calcification. Except for focal HV lesions in the intestinal submucosa of one diabetic patient, HV lesions were limited to the peritoneal membrane. None of the other extraperitoneal tissues showed such lesions. In conclusion, extraperitoneal vessels of PD patients show no relevant HV lesions when compared to peritoneal ones. This observation suggests that PD-related factors are the main contributors to the severity of vasculopathy. Uremia may participate in the development of the lesion but it does not seem to be responsible for its severity.

Keywords: Hyalinazing vasculopathy; Peritoneal dialysis; Peritoneum; Uremia

Introduction

Peritoneal dialysis (PD) treatment induces structural anomalies of the peritoneal membrane. Mesothelial cell loss, fibrosis, neoangiogenesis, and hyalinizing vasculopathy (HV) are common consequences of PD treatment [3,14,15]. Peritoneal vasculopathy was described in 1985

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by Gotloib et al. [5]. Conducting an ultrastructural study, they observed reduplication of the peritoneal submesothelial and vascular basal membrane, an observation very similar to that seen in diabetes. On light microscopy, the lesion is characterized by widening and hyalinization of the vessel wall. Further studies have demonstrated that the intensity of vasculopathy correlates with the time of dialysis, peritoneal fibrosis, and ultrafiltration loss [16]. The pathogenesis of HV is not fully understood. This is due, in part, to the difficulties of reproducing the lesion in experimental animal models. The high glucose content of the dialysis fluids, peritonitis episodes, and uremia have been considered etiological factors [1,4,5,16]. In a report of the biopsy registry study group, vasculopathy lesions were observed in the peritoneum in 28% of uremic, non-PD patients [16]. These lesions were less intense than those observed in long standing PD patients, but were similar to those seen in patients under short-term PD treatment (less than 24 months). A recent study describes minor lesions of vasculopathy in uremic, non-PD patients [15]. Other morphological studies showed no significant vasculopathy in the peritoneal samples of uremic, non-PD patients [2,6,8,13,14]. The lower prevalence and severity of vasculopathy in uremic patients, when compared to PD ones, suggest a greater etiological contribution of PD-related factors.

To further elucidate this hypothesis, we performed an autopsy study of seven PD patients who showed extensive and severe peritoneal HV lesions. The comparison of the degree of vascular lesions of peritoneal vessels with that of extraperitoneal vessels will help us to determine to which extent uremia contributes to the severity of HV. Uremia is a systemic condition that affects all tissues of the organism. Therefore, if uremia contributes significantly to the development of HV, such lesions should exist in extraperitoneal vessels. In this study, multiple organs and tissues were compared, placing special emphasis on other serosal surfaces such as the pleura and pericardium showing morphological and functional similarities with the peritoneum.

Materials and methods

Patients

From the pathology files of La Paz and Guadalajara University Hospitals, we selected seven autopsy cases of PD patients with prominent peritoneal HV lesions. In all cases, complete medical information was available. Clinicopathological features are shown in Table 1. The underlying renal disease was polycystic disease ($n = 2$), diabetes mellitus ($n = 2$), and glomerulonephritis ($n = 1$), but was unknown in two. Six patients were treated with continuous ambulatory and one with automatized PD. All of them received standard glucose-based dialysis fluids, with a mean treatment duration of 70.6 ($\pm$17.3) months.

Tissue processing and sample analysis

In all cases, a complete clinical autopsy following an "en bloc" removal method (Letulle technique) was performed [10]. From these cases, we selected histological slides from all the abdominal organs, heart and pericardium, lungs and visceral pleura, and central nervous system. In each autopsy case, a variable number of tissue samples (from 57 to 78), including extensive areas of visceral peritoneum, were present. Parietal peritoneum tissue was available in three cases. All samples were fixed in formalin and embedded in paraffin, and then cut into 3 μm sections and stained with hematoxylin-eosin. Histological analysis was carried out by two pathologists. Several data, including mesothelial status, submesothelial thickness, HV, inflammation, calcification, and fibrin deposits, were recorded. Grading of HV was performed using a method similar to that described by Honda et al. [6]. Four grades were used: grade 0, no abnormalities; grade 1, mild thickening without stenosis of the lumen; grade 2, moderate thickening with partial luminal stenosis; and grade 3, intense thickening with marked stenosis and luminal distortion or complete occlusion. To

Table 1. Clinical features of PD patients

Case	Age/gender	Time of PD (months)	Peritonitis episodes	Diabetes mellitus	MTC	Other
1	68/M	86	6	No	25.7/16.3	UF:600
2	62/F	88	3	No	22/15	UF failure (400)
3	77/F	74	2	No	20/13.8	UF failure, SP
4	50/F	48	na	Yes	na	–
5	78/M	53	0	Yes	14.3/7.7	UF:610
6	72/F	87	7	No	23.3/16.8	UF failure
7	65/M	58	na	No	na	–

na: not available, MTC: mass transfer coefficient, UF: ultrafiltration.

quantify the prevalence of normal vessels and different grades of vasculopathy in each autopsy case, 50 vessels were counted. Such an analysis was performed in the visceral peritoneum samples, and the results were expressed as percentages of vessels with normal appearance or grade 1, 2, and 3 vasculopathy.

Results

The histopathological features are summarized in Tables 2 and 3. HV lesions were intense and extensive in all PD patients. Except for one patient, obliterative, grade 3, vascular lesions were a common finding. Table 2 summarizes the percentages of normal vessels and those with different grades of vasculopathy from the visceral peritoneum. Other relevant pathological features are presented in Table 3. In consonance with vasculopathy, submesothelial fibrosis was intense, with a homogeneous hyaline quality. The mesothelial layer was absent in all samples. Two patients showed prominent inflammatory and fibrin deposits. Peritoneal calcifications were a relevant finding in two. The three parietal peritoneal samples showed pathological features similar to visceral ones, with prominent vasculopathy and fibrosis. Three PD patients had pericardial fibrosis that was intense in two of them. Two patients showed foci of pleural fibrosis in relation with bronchopneumonia. Regarding vascular pathology, the two patients with myocardial infarction showed coronary atherosclerotic lesions with associated total luminal thrombosis. Similar atherosclerotic lesions were seen in the aorta. Two patients with hypertension showed minor lesions of cerebral arteriolosclerosis. As expected, all atrophic kidneys revealed lesions of nephroangioesclerosis. In one of the PD diabetic patients, a focal area, located in the submucosa of the small bowel, showed lesions of vascular hyalinization equivalent to grade 1. Except for this patient and precise location, no other extraperitoneal HV lesions were observed. The pleura and pericardium, even in the fibrotic samples, showed no vascular lesions (Fig. 1).

Discussion

In this study, we have demonstrated that extraperitoneal vessels of PD patients showed no relevant HV lesions when compared to peritoneal ones. A similar observation was mentioned by Honda et al. in their study of peritoneal vasculature [6]. The authors suggest that PD-related factors, and not uremia, are the main contributors to the development of HV. Gotloib et al. were the first to describe the reduplication of the basement membrane of the peritoneal capillaries of PD patients [5]. In a subsequent study, Di Paolo and Sacchi confirmed such findings and observed similar lesions in diabetic, non-PD patients but not in non-diabetic,

Table 2. Percentage of normal and abnormal vessels from the visceral peritoneum

Case	Normal vessels (%)	Grade 1 (%)	Grade 2 (%)	Grade 3 (%)
1	50	13	20	17
2	42	21	27	10
3	55	20	5	20
4	62	17	13	8
5	33	23	29	15
6	62	15	15	8
7	53	25	22	0

Table 3. Pathologic features of PD autopsy cases

Case	Peritoneal acute inflammation	Peritoneal fibrin	Peritoneal calcification	Other
1	–	–	++	Pulmonary fibrosis, myocardial infarction
2	++	++	++	Intestinal hemorrhage
3	++	+	–	Ischemic colitis
4	–	–	–	Myocardial infarction
5	–	–	–	Intestinal hemorrhage
6	++	++	–	Aspergillus peritonitis
7	+	–	–	Atherosclerosis

–: absent, +: present, ++:prominent.

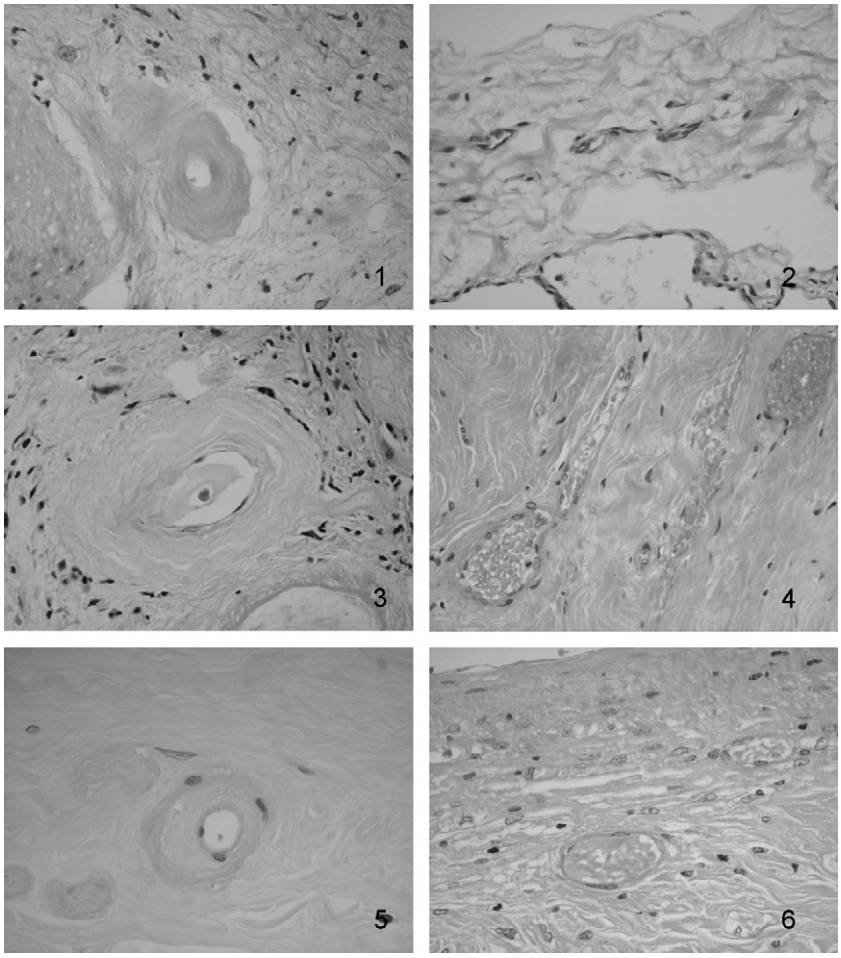

Fig. 1. Microphotographs 1, 3, and 5 show severe lesions of hyalinazing vasculopathy in the peritoneum of three different patients. Each image is compared with samples from normal (2), fibrotic (6), and pleura and pericardium (4) from the same patients. In clear contrast with peritoneal vessels, extraperitoneal vessels show no histopathological anomalies (hematoxylin-eosin, all images at the same magnification: × 150).

uremic patients [2]. Similar results were described by Pollock et al. in a series of PD and non-PD uremic patients [14]. Zweers et al. observed reduplicated basal membranes in peritoneal vessels from rats exposed to glucose-based PD-fluids [19]. However, no such lesions were present in rats exposed to glucose-free fluids. These rats were non-uremic, revealing that hypertonic glucose-based fluid was capable *per se* of basal membrane reduplication. All these studies suggested a capital role for glucose and related products in the development of HV. In the study conducted by Plum et al., vascular sclerosis was measured using a wall-to-lumen area ratio [13]. Following this method, vascular sclerosis was evident in PD patients. When compared to normal controls, an increased wall-to-lumen ratio was present in uremic, non-PD patients, but it was not statistically significant. A similar methodological approach was previously used by Mateijsen et al. [11]. They showed that peritoneal vessels from pre-dialysis patients had a lower ratio when compared to samples from PD patients, but in this study, no control group of normal, non-uremic patients was present. Honda et al. reported no vascular sclerosis lesions in the peritoneum of patients at the beginning of PD [7]. Similarly, in a previous study, we failed to detect vascular lesions in uremic patients, either at pre-dialysis or after hemodialysis treatment [8]. These results contrast with those reported by the peritoneal biopsy study group [16]. In a morphological study of numerous peritoneal biopsies, they found a greater prevalence of HV in uremic, non-PD patients when compared to normal controls. Methodological differences may partially explain the different results obtained. The biopsy processing and grading system used by Williams et al. seems to be more sensible for the detection of minor lesions. For light microscopy studies, they use semithin sections after tissue fixation with glutaraldehyde and formaldehyde and embedding in an acrylic resin. In contrast, all other studies use formaldehyde fixation and paraffin embedding. Subendothelial hyaline material determining lesions less than 7 μm in thickness may be difficult to detect by the latter standard tissue processing. The

grading system proposed by Honda et al. is similar to that of Williams et al. for the evaluation of moderate to intense lesions but differs in the measurement of slight ones. The grade 1 lesion proposed by Williams et al. can be interpreted as normal by other studies, including ours. Following the grading system of Honda et al., Sherif et al. [15] observed minor lesions of HV grade 1 in a group of 12 uremic, non-PD patients. In such a group, vessels showing grade 2 or 3 lesions were an exceptional finding.

In addition to glucose and related products, peritonitis has also been considered a major contributor to HV [4]. At least one of the patients of the present series, showing prominent HV, had no clinical episodes of peritonitis. It is important to remark that in non-renal patients, peritonitis is not associated with HV. Pathologists have the opportunity to study numerous abdominal surgical specimens showing peritonitis, and no lesions of HV are associated [9]. Samples such as those obtained from intestinal diverticular disease, inflammatory bowel disease, and hernia sacs show an inflammation pattern similar to that of PD (chronic sustained inflammation with superimposed acute episodes). It seems that in the absence of uremia or PD treatment, peritonitis "per se" is incapable of producing HV lesions. As for the vasculopathy seen in diabetes mellitus, advanced glycation end products (AGEs) seem to be responsible for the development of PD-related HV lesions [12]. AGEs accumulate with particular intensity in vessels with HV, and such deposit increases with the intensity of vasculopathy [7,12]. In addition to diabetes, uremia seems capable of inducing formation and tissue deposit of AGE [18]. Indeed, AGE accumulation in extraperitoneal vessels has been demonstrated in uremic patients [17]. Despite such deposition, and as stated by Honda et al. [6], HV lesions have never been found in any organs other than the peritoneum. From all these data, it seems that the major contributor to HV lesions must be related to PD fluid composition rather than uremia or peritonitis. Due to morphological and biochemical similarities to diabetic vasculopathy, glucose and related products must be considered the major causative agents.

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Capítulo 3. Evolución de la función peritoneal durante los primeros años de diálisis

6.1.3.1.- "Factores que influyen en los parámetros de transporte peritoneal durante el primer año en diálisis peritoneal: la peritonitis es el principal factor".

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Nephrology Dialysis and Transplantation 2005; 20: 1201-1206

Este trabajo responde a los objetivos 1 y 2

Factores que influyen en los parámetros de transporte peritoneal durante el primer año en diálisis peritoneal: la peritonitis es el principal factor.

Los estudios sobre la evolución de la función peritoneal durante el primer año en DP son escasos y sus resultados son contradictorios.

Objetivo: Analizar cuál es la evolución del transporte peritoneal y de la función renal residual (FRR) durante el primer año de tratamiento con DP y evaluar qué factores pueden influir en ella.

Métodos: Se estudiaron 249 pacientes tratados con DP continua ambulatoria tratados con soluciones de diálisis estándar (ricas en glucosa y en PDGs). La edad media de los pacientes era de 51.9±16 años, siendo 59 de ellos diabéticos. Se evaluó el transporte de pequeños solutos (mediante el MTC-U y MTC-Cr) y de agua (mediante la ultrafiltración neta), así como la FRR, basalmente y al año de diálisis.

Resultados: Durante el primer año en DP, la FRR (3.9±2.8 a 2.4±2.2 ml/min, $p<0.001$) y el MTC-U (22.7±7.8 a 20.7±6.6 ml/min, $p<0.001$) descendieron de forma significativa, mientras que la capacidad de UF aumentó significativamente (923±359 a 987±341 ml/4 h, $p<0.001$). El MTC-Cr disminuyó en dicho periodo, pero sin diferencias significativas (10.5±5.3 a 10.1±4.6 ml/min, ns). La evolución del transporte de pequeños solutos fue independiente de la edad, sexo, presencia de diabetes o dosis acumulada de glucosa hipertónica. Cuando se agruparon los pacientes según el MTC-Cr basal, se objetivó una tendencia a la normalización de los parámetros de función peritoneal.

Por otro lado, los pacientes con algún episodio de peritonitis durante el primer año en DP (n=88) mostraron un valor significativamente más elevado de MTC-

Cr al año que los pacientes sin peritonitis (11.1±5 frente a 9.5±4.2, p<0.01). La capacidad de UF descendió de forma no significativa en los pacientes con más de cuatro días acumulados de inflamación peritoneal (de 1062±447 a 1024±340 ml/4 h, ns), aumentando en el resto de pacientes.

Conclusiones: Los parámetros de transporte peritoneal tienden a normalizarse durante el primer año en DP, generalmente con un descenso del transporte de pequeños solutos y un aumento de la capacidad de UF. Esta tendencia es independiente de la edad, el sexo, la presencia de diabetes y de la mayor exposición a la glucosa de los líquidos de diálisis. El único factor independientemente asociado con los cambios en la función peritoneal fué la presencia de peritonitis durante el primer año en DP.

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Original Article

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Factors influencing peritoneal transport parameters during the first year on peritoneal dialysis: peritonitis is the main factor

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Abstract

Background. Studies on the evolution of peritoneal transport during the first year of peritoneal dialysis (PD) are scarce and their results are contradictory. The aim of the present study was to analyse the evolution of peritoneal transport and residual renal function during the first year on PD, and to determine the factors that may influence them.

Methods. We studied 249 patients on continuous ambulatory PD with glucose exchange solutions (117 men, 132 women, mean age 51.9 ± 16 years) 59 of whom had diabetes (25 type I). At baseline and after 1 year, we determined the mass transfer coefficients of urea (U-MTAC) and creatinine (Cr-MTAC), net ultrafiltration and residual renal function.

Results. Residual renal function decreased significantly during the first year (from 3.9 ± 2.8 to 2.4 ± 2.2 ml/min, $P < 0.001$). Both U-MTAC and Cr-MTAC decreased after 1 year [U-MTAC from 22.7 ± 7.8 to 20.7 ± 6.6 ml/min ($P < 0.001$), Cr-MTAC from 10.5 ± 5.3 to 10.1 ± 4.6 ml/min (NS)]. The ultrafiltration capacity increased significantly (from 923 ± 359 to 987 U 341 ml/4 h, $P < 0.001$). The evolution of MTAC values was independent of age, sex, diabetes and amount of hypertonic glucose used. When patients were grouped according to their initial Cr-MTAC, we observed a tendency toward normalization of the parameters of peritoneal function. Patients with peritonitis ($n = 88$) showed a first year increase in Cr-MTAC, which was significantly higher than in patients without peritonitis (11.1 ± 5 *vs* 9.5 ± 4.2, $P < 0.01$). Ultrafiltration decreased in patients with more than four accumulated days of peritonitis (from 1062 ± 447 to 1024 ± 340 ml/4 h, NS); it increased in patients without peritonitis.

Conclusions. The peritoneal transport parameters tended toward normalization during the first year on PD, mainly with a decrease of small solute transport and an increase of ultrafiltration capacity. This evolution is independent of age, gender, diabetes and higher exposure to glucose in PD solutions. Peritonitis was the only independent factor that affected peritoneal function during the first year on peritoneal dialysis.

Keywords: peritoneal dialysis; peritoneal transport; peritonitis; ultrafiltration

Introduction

Water and small solute transport in peritoneal dialysis (PD) patients changes with time on PD, and varies between individuals. Approximately 80% of patients on PD maintain stable peritoneal function. Some long-term PD patients show an increase in the peritoneal transport of small molecules and a decrease of their ultrafiltration (UF) rates [1,2]. Few studies have been focused on the changes of peritoneal transport during the first year on PD, and the available results are controversial. Some authors have found an initial increase of peritoneal transport of small solutes and a concomitant decrease of UF during the first year [3,4], while others have described a significant decrease in solute transport during the first months on PD [5].

It has been reported that a high peritoneal transport when starting PD determines higher medium- to long-term morbidity and mortality [6–8], but here too there is still some controversy. Some authors [9] believe that volume status in the context of impaired UF capacity, but not a high transport status, is responsible for the diminished technique and patient survivals.

The aims of the present study were: (i) to analyse the evolution of peritoneal transport parameters during the

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first 12 months on continuous ambulatory peritoneal dialysis (CAPD) and to investigate the factors related to it; and (ii) to study the course of patients with a high transport or low UF rate, or both, at baseline.

Subjects and methods

We evaluated 382 patients who were treated by PD between 1980 and 2001. Of them, 16 were censored because of renal transplantation ($n=10$), transfer to haemodialysis ($n=4$), death ($n=1$) or recovery of renal function ($n=1$) during the first year of follow-up. Additional patients were not included in the final analysis because a kinetic study at 12 months was not possible in them ($n=62$), or because they were treated with icodextrin during the first year on PD ($n=55$). In all, we analysed 249 patients who had at least two peritoneal transport studies: at baseline (between 15 and 45 days after the initiation of CAPD) and after 12 months on CAPD.

We performed a peritoneal transport kinetic study, consisting of a 4 h dwell time glucose exchange, and the taking of six peritoneal effluent samples (at 0, 30, 60, 120, 180 and 240 min) and one blood sample to calculate the peritoneal mass transfer area coefficient of urea (U-MTAC, ml/min) and creatinine (Cr-MTAC, ml/min) using a previously described [10] mathematical model. This coefficient represents the isolated diffusive capacity of the membrane under theoretically infinite dialysate flow. The patients fasted during each functional study, and they received no drugs except low doses of subcutaneous insulin, if necessary. The kinetic study was done at least 30 days after any episode of peritonitis. The presence of peritoneal inflammation was determined by performing cell counts on the peritoneal effluent, and peritonitis was defined as the presence of >100 white blood cells/μl with >50% polymorphonuclear cells. Net UF rate (ml) was estimated by the net negative balance (weighing the bag after drainage), using a 2 l 3.86% glucose exchange during 4 h of dwell time. This value represents mostly the convective transport capacity. Residual renal function (RRF, ml/min) was estimated by the average of the renal urea and creatinine clearances.

In order to distinguish the evolution of the different parameters according to the type of peritoneal transport at baseline, we established five transport categories, according to the quintile distribution of the patients' initial Cr-MTAC, as follows: 1st quintile, Cr-MTAC <6.7; 2nd quintile, Cr-MTAC 6.7–8.6; 3rd quintile, Cr-MTAC 8.61–10.7; 4th quintile, Cr-MTAC 10.71–13.5; and 5th quintile, Cr-MTAC >13.5. The correspondence between creatinine peritoneal equilibration test and MTAC quintiles is as follows: low transporters (<6.7 ml/min), low-average (6.71–8.6 ml/min), high-average (8.61–10.7 ml/min) and high transporters (>10.71 ml/min). For the last group, we distinguish between high and very high (>13.5 ml/min) in order to establish differences that facilitate the analysis.

In addition, we analysed separately the patients who had UF failure at baseline ($n=12$), as defined by Ho-dac-Pannekeet *et al.* [11]. The amount of glucose exchange solutions used was registered every month, and then it was averaged for the entire year. Excess use of hypertonic glucose was defined as the use of >25% of the total volume of dialysate of 3.86% glucose exchange solutions per day or >50% of 2.27% glucose solutions per day.

Statistical analysis

Values are expressed as the mean ± SD, and a $P<0.05$ was considered statistically significant. The χ^2 test was used to compare proportions, and the Student *t*-test to compare means. A two-way repeated measures analysis of variance (ANOVA) was used. A '*post hoc*' test was performed using the Bonferroni approach.

Results

All patients

We studied 249 patients (47% men, 53% women) with a mean age of 51.9 ± 16 years (range 10–85). Of them, 24% had diabetes (25% of them type I diabetes mellitus). Their primary kidney diseases were: diabetic nephropathy in 54 patients, tubulo-interstitial nephritis in 50, chronic glomerulonephritis in 36, nephroangiosclerosis in 34, systemic in 25, polycystic renal disease in 19, vascular in seven, hereditary in three, other in two and unknown in nine. All patients were treated with CAPD and used glucose-based dialysis solutions during the study. Of the cohort, 34 patients (13.6%) used an excess of glucose dialysis solutions during the first year on CAPD. In all, 88 patients (35%) had some episode of peritonitis during their first year on CAPD, with a mean of 5.9 ± 4.6 days of peritoneal inflammation (range 1–25); 49 patients had >4 accumulated days of peritonitis during the study period. No patient received icodextrin.

During the first year on CAPD, the peritoneal transport of low molecular weight solutes decreased and the UF rate showed a significant increase (Table 1). There was an inverse linear correlation between Cr-MTAC and UF at the beginning of treatment ($r=-0.14$, $P=0.019$) and their values after 12 months ($r=-0.24$, $P=0.000$). No significant differences in the evolution of peritoneal transport were found when adjusted for age, gender or diabetes status. Men started CAPD with significantly higher U-MTAC than women (23.7 ± 8.3 *vs* 21.8 ± 7.2, $P=0.046$). Both sexes showed the same tendency in the evolution of peritoneal function parameters (data not shown). There were no differences between diabetic and non-diabetic patients in the evolution of peritoneal parameters: U-MTAC decreased in both groups (from 24.1 ± 7 to 22.4 ± 6 in

Table 1. The evolution of the parameters of peritoneal function during the first year on CAPD

	Baseline	12 months	*P*
Residual renal function (ml/min)	3.9 ± 2.8	2.4 ± 2.2	0.000
U-MTAC (ml/min)	22.7 ± 7.8	20.7 ± 6.6	0.000
Cr-MTAC (ml/min)	10.5 ± 5.3	10.1 ± 4.6	0.28
Ultrafiltration (ml/4 h)	923 ± 359	987 ± 341	0.001

U-MTAC = peritoneal mass transfer area coefficient of urea; Cr-MTAC = peritoneal mass transfer area coefficient of creatinine.

diabetics, and from 22.3 ± 8 to 20.2 ± 6 in non-diabetics, NS), as did Cr- MTAC (from 11 ± 5 to 10.5 ± 4 in diabetics, and from 10.3 ± 5 to 10 ± 4.7 in non-diabetics, NS). UF capacity increased both in diabetics (from 957 ± 349 to 995 ± 332) and in non-diabetics (from 913 ± 363 to 987 ± 343), but no significant differences were found between them.

Grouped according to initial Cr-MTAC

When we analysed the results according to the Cr-MTAC at baseline (Figure 1), we observed a regression-to-mean phenomenon of peritoneal transport parameters. Patients with MTAC-Cr >13.5 at baseline (5th quintile) showed a significant decrease of U-MTAC and Cr-MTAC. In contrast, patients with the lowest basal Cr-MTAC (1st quintile) had significant increases of both MTAC values after the first year. A significant increase of UF capacity and a decrease of RRF were found in all groups, with no differences between the different groups.

Patients with initial UF failure

Of the cohort, 12 patients (4.8%) started CAPD with UF failure and their UF capacity increased significantly after the first year on CAPD (from 312.5 ± 77.2 to 575 ± 246 ml, $P = 0.005$). Both U-MTAC (from 26.7 ± 8.9 to 21.1 ± 7.6, $P = 0.06$) and Cr-MTAC (from 15.1 ± 4.8 to 11.2 ± 4.5 ml/min, $P < 0.041$) decreased during this period, as did their RRF (from 4.5 ± 2.4 to 3.3 ± 2.7 ml/min, $P < 0.07$). Only two patients remained with UF failure at the end of the year.

Excess use of hypertonic glucose solutions

Except for a significantly higher decrease of RRF in those who used an excess of hypertonic glucose (from 4.3 ± 2.9 to 2.1 ± 1.6 *vs* 3.8 ± 2.7 to 2.4 ± 20.3, $P = 0.035$), we found no correlation between the excess use of hypertonic glucose solutions and the evolution of peritoneal function parameters.

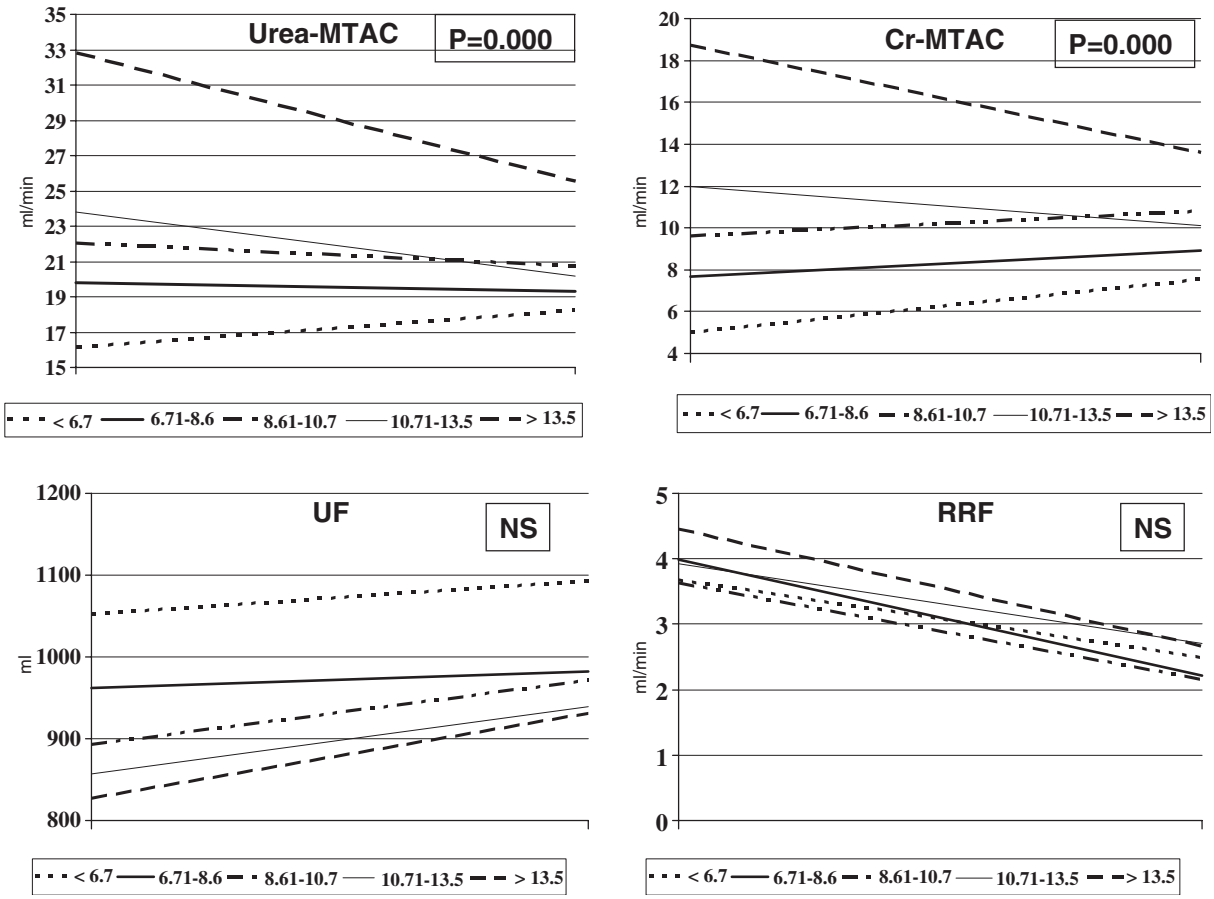

Fig. 1. The parameters of peritoneal function according to the initial Cr-MTAC category. 1st quintile, Cr-MTAC <6.7; 2nd quintile, Cr-MTAC 6.71–8.6; 3rd quintile, Cr-MTAC 8.61–10.7; 4th quintile, Cr-MTAC 10.7–13.5; and 5th quintile, Cr-MTAC >13.5. The *P*-value expresses the differences between groups in the comparison of the gradients. *Post hoc* analysis of U-MTAC showed statistically significant ($P < 0.05$) differences when each subgroup is compared with each of the others, except for 1st *vs* 2nd quintile, 2nd *vs* 3rd, 2nd *vs* 4th and 3rd *vs* 4th. *Post hoc* analysis of Cr-MTAC showed statistically significant differences ($P < 0.05$) when each subgroup is compared with each of the others, except for 3rd *vs* 4th quintile.

Influence of peritonitis

The characteristics of patients with and without peritonitis are shown in Table 2. The evolution of U-MTAC and RRF was not significantly different in patients with or without peritonitis during the first year on CAPD (Figure 2). The Cr-MTAC decreased in the patients without peritonitis, while the patients who had some episodes of peritonitis showed an increase of Cr-MTAC, which was higher in patients with >4 accumulated days of peritonitis. These differences were not statistically significant. However, there was a significant difference in Cr-MTAC values after the first year between the patients with and without peritonitis (11.1 ± 5 *vs* 9.5 ± 4.2, $P < 0.01$). The patients with >4 days of peritonitis showed no significant change in UF capacity, in contrast to the increase of UF capacity found in patients who had <4 days of, or no, peritonitis.

Table 2. The characteristics of patients with and without peritonitis

	Peritonitis ($n = 88$)	No peritonitis ($n = 161$)	*P*
Age (years)	52.7 ± 14	51.6 ± 16	NS
Gender (M/F)	44%/56%	48%/52%	NS
Diabetes	22.7%	24.4%	NS
Excess use of hypertonic glucose	22.7%	12.4%	<0.05

Discussion

During the first year on CAPD, we found a tendency for low molecular weight solute transport to decrease and UF capacity to increase. UF failure is one of the major causes of drop-outs among PD patients. It has been reported that some long-term PD patients lose UF capacity and develop an increase in small solute transport [3,5]. Those changes in peritoneal kinetics appear after the second or third years of treatment. However, the peritoneal membrane function of most patients remains stable with time on PD [1,2]. In the study by Selgas *et al.* [1], 80% of patients showed mild or no changes in peritoneal function during PD. Blake *et al.* [2] observed that >70% of patients maintain a stable peritoneal function at 12 months, while only 3% show a decrease in D/P creatinine. Differing follow-ups

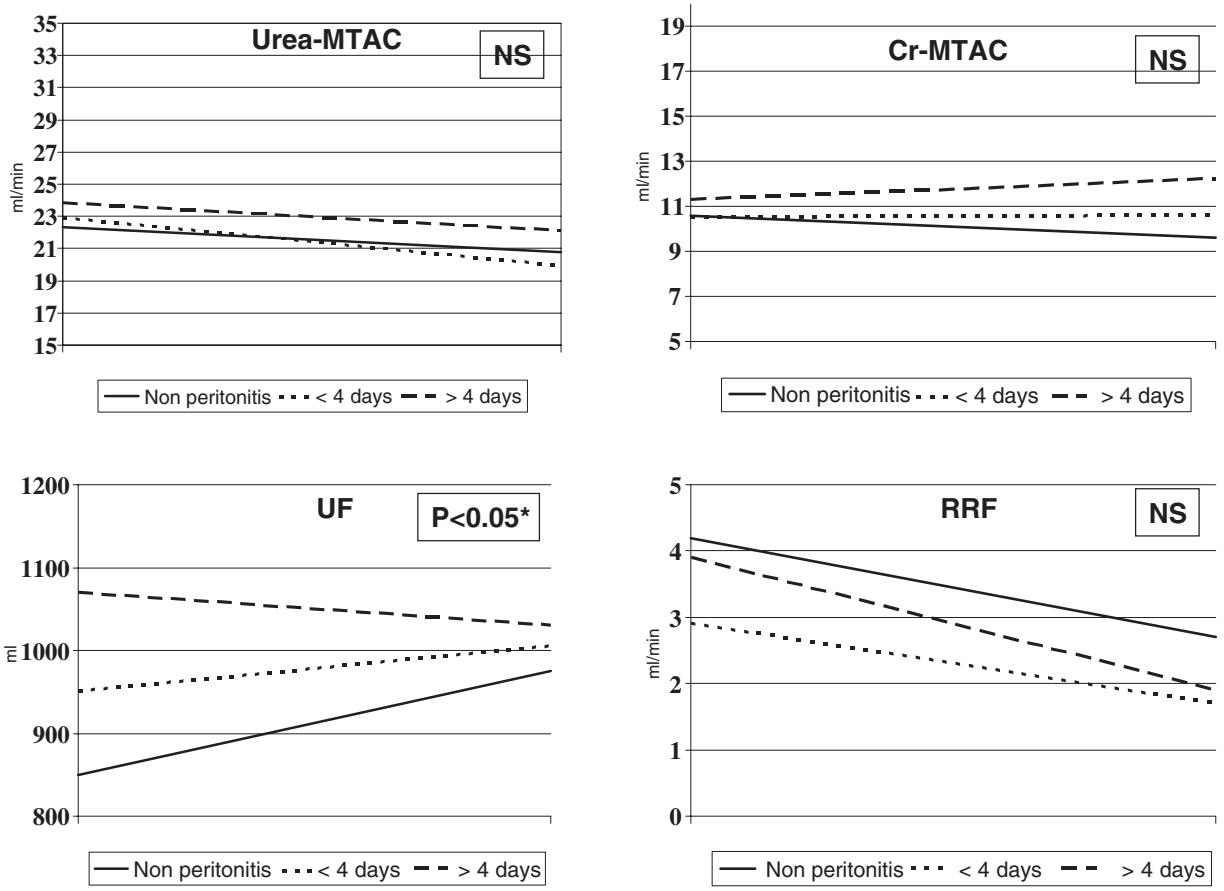

Fig. 2. The parameters of peritoneal function of patients without peritonitis, and of those with less, or more, than 4 days of peritonitis. The *P*-value expresses the differences between groups in the comparison of the gradients. None of the parameters showed significant differences when patients with and without peritonitis were compared. *$P = 0.037$ when comparing patients with <4 days of peritonitis with those with a total of >4 days of peritonitis.

and variability in the definition of peritoneal membrane failure may be the reasons for these discrepancies. The results of short-term studies are still controversial. Our findings agree with those of some previous studies [5,12], but conflict with others [2–4]. Struijk *et al*. [5] reported that, 4 months after the beginning of PD, the patients showed lower net UF and higher small solute transport than in the first month. In contrast, Davies *et al*. [3] found a significant increase of D/P creatinine, associated with a loss of UF capacity after 6 months on PD, and a stabilization of parameters of peritoneal function during the next 30 months. Chung *et al*. [4] have described the initial increase in D/P creatinine and the concomitant decrease in net UF after the first year on PD in >70% of patients. All these studies have employed the D/P creatinine ratio to analyse peritoneal transport kinetics, a less sensitive marker than the MTAC value used in our study. In addition, we analysed the evolution over time of peritoneal transport separately, according to its baseline status. When we took into account the initial Cr-MTAC, we found a trend towards a normalization of peritoneal transport parameters during the first 12 months on CAPD, independently of the initial status. Moreover, the group of patients with the highest transport at the beginning of treatment (Cr-MTAC >13.5) showed a significant decrease of small molecule transport and also an increase of UF capacity during the first year on CAPD. This may indicate an initial adaptation of the peritoneal membrane to PD fluids with a decrease of vascular surface area. Davies *et al*. [13] have previously reported that longitudinally the changes over time in solute transport vary according to the initial status of transport. They observed that the increase in solute transport occurs mainly in patients with low transport at baseline. Lo *et al*. [12] obtained similar results. They found a decrease in solute transport in high transport patients, but an increase in initially low transporters. The higher rate of drop-outs from PD among high transporters has been proposed as the possible reason for the tendency of small solute transport to increase.

There is agreement on the fact that the loss of UF capacity increases with time on PD. The main factor associated with the decrease of UF capacity is the increase in small solute transport. As shown in Figure 1, the patients from all groups showed a significant increase in UF capacity during the first 12 months, independently of their type of peritoneal transport at baseline. In addition, the patients with UF failure at baseline (UF <400 ml/4 h 3.86%) also showed a significant increase of UF capacity during the year, with only two patients remaining with UF failure after the year. We have found that significant inter-patient differences exist, but we believe that a water transport abnormality manifest when beginning PD does not always presage subsequent UF failure. However, longer follow-ups of these patients are needed to confirm our findings. An adaptative process during the early stages of the treatment takes place in PD patients, with a decrease or increase of peritoneal surface area, depending on the number of capillaries perfused during the contact of dialysis solutions with the peritoneal membrane. Our results lead us to hypothesize that the first year is a stage during which adaptation occurs to the vasoactive processes that occur during PD. The PD patients whose UF failure persisted after the first year are those with inherent UF failure, and they reflect the true inability of the peritoneum to adapt, in their cases, to the glucose of the dialysate.

When we analysed the possible causes of the changes in peritoneal transport, we analysed the main risk factors described, such as chronic exposure to bioincompatible dialysis solutions and peritonitis. Since we included in the study only patients treated with glucose exchange solutions, a selection bias cannot be excluded. Glucose has been described as the main cause of peritoneal damage [14]; however, a vicious circle exists, because higher solute transport induces higher use of hypertonic glucose exchange solutions, and this also induces higher small solute transport. In the present study, the amount of glucose used was not related to changes in peritoneal transport during the first year on CAPD. It is possible that the follow-up was too short to reveal the functional changes that are expected to occur after prolonged exposure to high concentrations of glucose. In contrast to our findings, Davies *et al*. [15] have found that early exposure to large amounts of hypertonic glucose solutions preceded the functional abnormalities of the peritoneum in long-term PD patients. Previous studies reported by Selgas *et al*. [16] showed that the patients who developed early UF failure had a higher prevalence of diabetes and also had larger glucose loads since their second years on PD than the patients who did not. Similar findings have been reported by Chung *et al*. [4], with a direct correlation between the amount of glucose in the dialysate and changes in peritoneal transport. In our series, water and small solute transport showed no correlation with diabetes status. It has been stated that diabetics have higher solute transport [17,18] in association with a higher effective surface area [19], but in the present study whether or not a patient had diabetes did not influence the subsequent evolution of peritoneal transport. This may indicate only that diabetic changes do not appear early in the evolution. However, diabetics showed higher initial RRF and higher loss of RRF during the first year on CAPD, a loss that may be due to their starting on PD earlier.

In our series, the most important factor influencing the short-term peritoneal kinetics was the presence of peritonitis, which during the first year on CAPD had a deleterious effect on the peritoneal transport of water and small solutes. The patients with some peritonitis, in contrast to those without, showed an increase of Cr-MTAC. This indicates that, in the early stages of PD, inflammation may have induced structural changes in the peritoneum, such as an increase of the effective vascular surface area, which alters peritoneal small solute transport. In contrast, the impact of

peritonitis on water transport was only detected in patients with >4 accumulated days of peritonitis. These findings concur with those obtained in long-term PD patients [3,10]. Blake *et al.* [2] previously have reported the association between peritonitis and an increase of peritoneal transport. Fußholler *et al.* [20] have also found that patients with histories of peritonitis showed an increased small solute transport. However, when they considered the incidence of peritonitis, no significant differences were found when comparing that group with those who had no peritonitis. In addition, as previously described [3], we too have found that the severity of peritoneal inflammation shows a direct relationship to the degree of the functional alteration.

In summary, there is a tendency toward normalization in peritoneal transport parameters during the first year on CAPD, mainly a decrease of small solute transport and an increase of UF capacity. These initial peritoneal changes are mainly influenced by peritonitis, but not by an early exposure to higher amounts of glucose in PD solutions.

Conflict of interest statement. None declared.

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Capítulo 4. Alteraciones morfológicas de la membrana peritoneal asociadas con la función peritoneal durante los primeros años de diálisis

6.1.4.1.-"La conversión mesenquimal de las células mesoteliales como mecanismo responsable del elevado transporte de solutos en diálisis peritoneal: papel del factor de crecimiento del endotelio vascular".

Aroeira LS, Aguilera A, Selgas R, Ramírez-Huesca M, Pérez-Lozano ML, Cirugeda A, Bajo MA, del Peso G, Sánchez-Tomero JA, Jiménez-Heffernan JA, López-Cabrera M.

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Este trabajo responde a los objetivos 2 y 3

La conversión mesenquimal de las células mesoteliales como mecanismo responsable del elevado transporte de solutos en diálisis peritoneal: papel del factor de crecimiento del endotelio vascular.

Durante la DP, la membrana peritoneal está expuesta a soluciones de diálisis bioincompatibles que provocan transición epitelio-mesenquimal (TEM) de las células mesoteliales, fibrosis y angiogénesis. El fallo de ultrafiltración se asocia a alto transporte de pequeños solutos y a incremento de la superficie vascular peritoneal, lo que sugiere implicación del factor de crecimiento del endotelio vascular (VEGF). En pacientes en DP, la fuente de VEGF no está clara.

Objetivo: Analizar la correlación de la TEM mesotelial con los niveles de VEGF y con la función peritoneal.

Métodos: Se aislaron células mesoteliales del efluente peritoneal de 37 pacientes en DP y se analizó su conversión epitelio-mesenquimal. Para estudiar la función peritoneal se utilizó el coeficiente de transferencia de masas de creatinina (MTC-Cr). La concentración de VEGF se midió mediante el procedimiento estándar. Además, en biopsias peritoneales de 12 pacientes en DP y 6 controles sanos se realizó un análisis inmunohistoquímico para evaluar la expresión de VEGF y citoqueratinas.

Resultados: Las células mesoteliales de efluente peritoneal con morfología no epitelioide producían *ex vivo* mayor cantidad de VEGF que las células mesoteliales con morfología epitelial ($p<0.001$). Los pacientes que presentaban en el efluente células de morfología no epitelioide tenían mayores niveles séricos de VEGF que los que mostraban células mesoteliales de morfología epitelial ($p<0.01$). La producción *ex vivo* de VEGF por las células mesoteliales

de efluente se correlacionó con los niveles séricos de VEGF ($r=0.6$, $p<0.01$). Además, el MTC-Cr se correlacionó con los niveles de VEGF en cultivo ($r=0.8$, $p<0.001$) y en suero ($r=0.35$, $p<0.05$). El MTC-Cr se asoció también con el fenotipo mesotelial. En las biopsias peritoneales de pacientes con alto transporte peritoneal se observó expresión simultánea de VEGF y marcadores mesoteliales en células fibroblásticas del estroma.

Conclusiones: Las células mesoteliales derivadas de la TEM mesotelial son la principal fuente de VEGF en pacientes en DP, y pueden ser responsables del alto transporte peritoneal.

Mesenchymal Conversion of Mesothelial Cells as a Mechanism Responsible for High Solute Transport Rate in Peritoneal Dialysis: Role of Vascular Endothelial Growth Factor

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• ***Background:*** **During peritoneal dialysis (PD), the peritoneum is exposed to bioincompatible dialysis fluids that cause epithelial-to-mesenchymal transition of mesothelial cells, fibrosis, and angiogenesis. Ultrafiltration failure is associated with high transport rates and increased vascular surface, indicating the implication of vascular endothelial growth factor (VEGF). Sources of VEGF in vivo in PD patients remain unclear. We analyzed the correlation between epithelial-to-mesenchymal transition of mesothelial cells and both VEGF level and peritoneal functional decline.** ***Methods:*** **Effluent mesothelial cells were isolated from 37 PD patients and analyzed for mesenchymal conversion. Mass transfer coefficient for creatinine (Cr-MTC) was used to evaluate peritoneal function. VEGF concentration was measured by using standard procedures. Peritoneal biopsy specimens from 12 PD patients and 6 controls were analyzed immunohistochemically for VEGF and cytokeratin expression.** ***Results:*** **Nonepithelioid mesothelial cells from effluent produced a greater amount of VEGF ex vivo than epithelial-like mesothelial cells ($P < 0.001$). Patients whose drainage contained nonepithelioid mesothelial cells had greater serum VEGF levels than those with epithelial-like mesothelial cells in their effluent ($P < 0.01$). VEGF production ex vivo by effluent mesothelial cells correlated with serum VEGF level ($r = 0.6$; $P < 0.01$). In addition, Cr-MTC correlated with VEGF levels in culture ($r = 0.8$; $P < 0.001$) and serum ($r = 0.35$; $P < 0.05$). Cr-MTC also was associated with mesothelial cell phenotype. VEGF expression in stromal cells, retaining mesothelial markers, was observed in peritoneal biopsy specimens from high-transporter patients.** ***Conclusion:*** **These results suggest that mesothelial cells that have undergone epithelial-to-mesenchymal transition are the main source of VEGF in PD patients and therefore may be responsible for a high peritoneal transport rate.** ***Am J Kidney Dis*** **46:938-948.**

INDEX WORDS: Peritoneal dialysis (PD); mesothelial cells; peritoneal transport rate; vascular endothelial growth factor.

PERITONEAL DIALYSIS (PD) is an alternative to hemodialysis (HD) for the treatment of patients with end-stage renal disease. In PD patients, the peritoneal membrane acts as a permeability barrier across which ultrafiltration and diffusion take place.[1] Unfortunately, long-term exposure to bioincompatible dialysis solutions and repeated episodes of peritonitis or hemoperitoneum cause injury of the peritoneum, which progressively undergoes fibrosis and angiogenesis and, ultimately, ultrafiltration failure.[2,3] The peritoneal membrane is lined by a monolayer of mesothelial cells (MCs) that have characteristics of epithelial cells and secrete various substances involved in homeostasis of the peritoneum.[4] For a long time, MCs have been considered mere victims of peritoneal injury during PD, whereas resident peritoneal fibroblasts classically have been considered mainly responsible for structural and functional peritoneal alterations.

Recently, we showed that soon after PD therapy is initiated, peritoneal MCs from dialysis effluent show a progressive loss of epithelial phenotype and acquire mesenchymal characteristics.[5] In immunohistochemical studies of peritoneal biopsy specimens from PD patients, we showed the

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expression of mesothelial markers in stromal α-smooth muscle actin–positive myofibroblasts, suggesting that these cells stemmed from local conversion of MCs.[5,6] Recently, myofibroblastic conversion of MCs was confirmed in vivo by injection of an adenovirus vector that transferred active transforming growth factor β1 in rat peritoneum.[7] In addition, we and others showed that this mesenchymal transformation of MCs can be induced in vitro with various stimuli.[2,5,8]

These biochemical and morphological MC changes are reminiscent of those that take place during epithelial-to-mesenchymal transition (EMT).[9] EMT is a complex process that starts with the disruption of intercellular junctions and loss of apical-basolateral polarity typical of epithelial cells, which then are transformed into fibroblast-like cells with pseudopodial protrusions and increased migratory, invasive, and fibrogenic features.[9] Our findings suggest that new fibroblasts may arise from local conversion of MCs by EMT during repair responses of the peritoneal tissue induced by PD.[5,6] A portion of these fibroblast-like MCs are released into PD fluid, and a portion of the remaining cells invade the submesothelium stroma because of their increased migratory/invasive capacity and may contribute to PD-induced fibrosis of the peritoneum.[5] Fibroblast-like MCs may retain a permanent mesenchymal state as long as initiating stimuli persist.

However, fibrosis is not the unique structural alteration in the peritoneal membrane induced by PD. In parallel with this alteration, the peritoneum shows a progressive increase in capillary number (angiogenesis) and vasculopathy.[3,10] There is evidence that angiogenesis and augmented vessel permeability are the main determinants of increased solute transport across the peritoneal membrane and ultrafiltration failure.[2,3] Vascular endothelial growth factor (VEGF) is a potent proangiogenic factor involved, among other molecules, in endothelial cell proliferation and vascular permeability.[11] It was proposed that local VEGF production during PD has a central role, in conjunction with other proangiogenic factors, in processes leading to peritoneal angiogenesis and functional decline.[12-14] Although MCs produce VEGF in vitro in response to various stimuli,[15-18] the main source of VEGF in PD patients and mechanisms implicated in VEGF upregulation during PD remained unclear. Here, we show that nonepithelioid MCs produce much greater amounts of VEGF than epithelial-like MCs, and patients who drain nonepithelioid MCs in their effluent have greater serum VEGF levels than those draining epithelial-like MCs. In addition, we show that peritoneal transport rates correlate with VEGF levels, ex vivo and in vivo, and MC phenotype. A mechanism responsible for high peritoneal solute transport rate based on EMT of MCs is proposed.

METHODS

Patients

We included 37 clinically stable patients on PD therapy; 18 patients on continuous ambulatory PD and 19 patients on automated PD therapy, 27 men and 10 women ranging in age from 25 to 79 years (mean, 61.7 $\pm$ 14.5 years). Mean time on PD therapy was 12.6 $\pm$ 15.5 months (range, 3 to 62 months). Causes of renal failure were nephrosclerosis in 10 patients, glomerulonephritis in 8 patients, diabetes in 5 patients, chronic pyelonephritis in 5 patients, polycystic kidney disease in 4 patients, unknown cause in 3 patients, and other causes in 2 patients. Twenty-five patients received peritoneal solution based on different glucose concentrations: 1.36%, 75.5 mmol/L; 2.27%, 126 mmol/L; and 3.86%, 214.3 mmol/L and lactate, and 12 of these patients received 1 exchange/d with icodextrin-containing solution. Eight patients were treated with solution containing glucose degradation product–free glucose and lactate; and 4 patients, with solution containing glucose and bicarbonate. Most patients (32 of 37 patients) were administered recombinant human erythropoietin during the study. Duration of active peritoneal inflammation is defined as time (days) from the start of peritonitis (elevation in cell count in PD effluent) and normalizing of cell count. Six patients showed peritonitis, and hemoperitoneum was seen in 4 patients. Mean time of active peritoneal inflammation in these 6 patients was 6.2 $\pm$ 5.54 days (range, 3 to 16 days); 2 patients were from the epithelial-like group (range, 4 to 5 days), and 4 patients, from the nonepithelioid group (range, 3 to 16 days).

Peritoneal glucose load was calculated as the sum of glucose contained in each PD fluid bag during the entire time on PD therapy (months). As control groups, 24 patients on HD therapy and 15 young healthy volunteers also were included.

The present study was approved by the Ethics Committee of Hospital Universitario de la Princesa (Madrid, Spain). Written consent was obtained from PD patients included in the study to use serum and effluent samples. Oral informed consent was obtained from omentum donors before elective surgery.

Measurement of Peritoneal Transport Rate

Urea (urea-MTC) and creatinine mass transfer coefficients (Cr-MTC) were measured by using a standard method. Ultrafiltration capacity is defined as a peritoneal exchange of 4 hours using 3.86% (214.3 mmol/L) glucose. Type I perito-

neal membrane failure is defined as a Cr-MTC greater than 11 mL/min and ultrafiltration less than 400 mL/4 hours.[19]

Isolation and Culture of MCs

MCs from PD patients were obtained from PD effluent by using the method described previously.[20] MCs were cultured in Earle M199 medium (Biological Industries, Ashrat, Israel) supplemented with 20% fetal calf serum (Gibco BRL; Life Technologies, Paisley, Scotland), 50 IU/mL of penicillin, 50 μg/mL of streptomycin (ICN Biomedicals, Costa Mesa, CA), and 2% Biogro-2 (Biological Industries).

We previously described that confluent MC cultures from PD effluent showed 3 different phenotypes: epithelial-like, similar to omentum-derived MCs; transitional; and fibroblast-like MCs that remained stable for 2 to 3 cell passages. Frequencies of those MC cultures were approximately 54%, 24%, and 17%, respectively. We also described a less frequent (5%) cell population with mixed phenotypes.[5] Given that transitional and fibroblast-like MCs were similar in terms of molecular expression,[5] in this study, we grouped them into a single category. Therefore, confluent MC cultures were classified according to cellular morphological characteristics and extracellular matrix component expression into 2 groups: epithelial-like (n = 23) and nonepithelioid (n = 14). We did not obtain MC cultures with mixed phenotypes in this instance. The purity of effluent-derived MC cultures was determined by the expression of standard mesothelial markers: intercellular adhesion molecule 1, cytokeratins, and calretinine.[5]

Human peritoneal MCs used as control cells were obtained from omentum samples collected from consenting nonuremic patients undergoing elective abdominal surgery by using the method described by Stylianou et al.[21] To induce transdifferentiation in vitro, human peritoneal MCs were treated with human recombinant transforming growth factor β (0.5 ng/mL) and interleukin 1 (2 ng/mL; R&D System, Minneapolis, MN), as described in our previous study.[5]

The purity of omentum-derived MC cultures was determined by the expression of standard mesothelial markers: intercellular adhesion molecule 1, cytokeratins, and calretinine.[5] These MC cultures were negative for von Willebrand factor, excluding endothelial cell contamination.

Serum Sample Collection

Serum samples were obtained from PD patients at the same times that effluent MCs were collected. Serum samples from HD patients were obtained before the first HD session of the week. All samples were obtained by using vacutainer systems (Becton & Dickson, Frankling Lakes, NJ). After coagulation, samples were centrifuged and sera were collected and stored at –80°C for posterior analysis.

Western Blot Analysis

For Western blotting, first-passage MC cultures were lysed in buffer (1% sodium deoxycholate, 0.1% sodium dodecyl sulfate), and total protein was quantified by using a total-protein assay kit (Pierce, Cambridge, MA). MC proteins (50 μg) were resolved in 8% to 10% sodium dodecyl sulfate-polyacrylamide gels. Proteins were transferred to nitrocellulose membranes, which were blocked with fat-free milk and incubated with specific collagen type I, collagen type IV, and fibronectin monoclonal antibodies (Sigma-Aldrich, San Luis, MO). Membranes were incubated with goat antimouse immunoglobulin G antibody conjugated with peroxidase (Pharmigen, San Diego, CA) and developed with an enhanced chemiluminescence detection kit (Amersham Biosciences, Freiburg, Germany). Blot images were acquired with an LAS-1000 Charge Coupled Device camera (Fujifilm, Cedex, France).

VEGF Measurements

For VEGF concentration analysis, media of confluent MC cultures in the first passage were replaced, and 18 hours later, supernatants were collected and stored at –80% until analysis. Sera and MC supernatant VEGF concentrations were assessed by means of a standard enzyme-linked immunoassay kit (Quantiokine; R&D System). Results of VEGF concentrations in supernatants were normalized with total protein of cell lysate and reported as picograms per microgram. Results of serum concentrations are reported as picograms per milliliter.

Biopsy Processing and Immunohistochemical Analysis

In our nephrology departments, we routinely obtain peritoneal parietal biopsy specimens from PD patients during peritoneal catheter insertion or removal, elective surgeries (inguinal hernia sac, nephrectomy), and renal transplantation. Twelve PD patients included in this study were selected according to their peritoneal transport characteristics and subdivided into 2 groups: low-normal (Cr-MTC range, 4 to 7.2 mL/min; n = 6) and high (range, 12 to 15.2 mL/min; n = 6) transporters. In addition, normal control samples (n = 6) of parietal peritoneum from nonuremic patients who underwent elective surgery also were included in this study. Written consent was obtained from patients before we obtained the peritoneal samples.

To avoid mesothelial artifactual detachment, peritoneal samples were carefully manipulated and immediately fixed with neutral buffered 3.7% formalin (pH 7.3) for 12 to 24 hours. While immersed in formalin, they were attached gently to a flat surface to avoid retraction. Afterward, samples were cut and embedded in paraffin and cut into 3-μm sections. Paraffin sections were mounted on precoated slides, routinely deparaffinized and rehydrated, and incubated with 3% hydrogen peroxide in methanol to block endogenous peroxidase activity. Antigen retrieval was performed by using a citric acid solution (pH 6) heated with a pressure cooker. Indirect immunohistochemical studies were performed in serial sections from the same peritoneal samples, using anti-VEGF polyclonal antibody (Zymed, San Francisco, CA) and antipancytokeratin monoclonal antibody (Dako, Glostrup, Denmark), as described elsewhere.[6] VEGF and cytokeratin expression was recorded by using a semiquantitative scale described previously.[6]

Statistical Analysis

Results are given as mean ± SD, whereas median and range were used for such non–normally distributed parame-

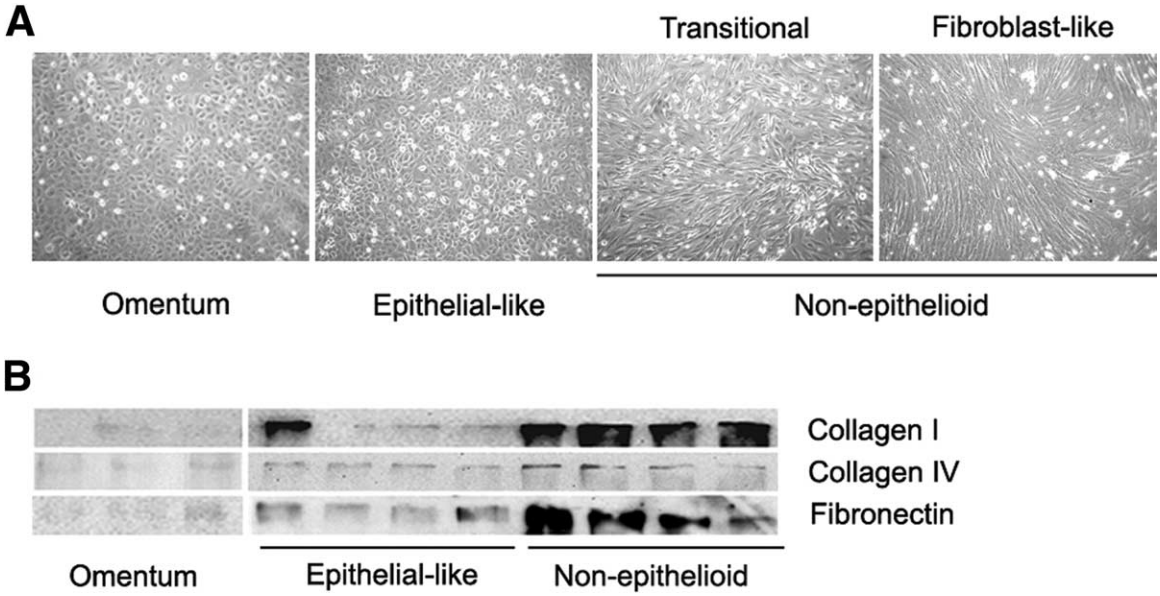

Fig 1. (A) Phase-contrast microscopy shows different morphological characteristics of MCs: omentum, epithelial-like, transitional, and fibroblast-like. Transitional and fibroblast-like MCs were grouped as a single category termed nonepithelioid MCs. (B) Western blot showing expression of extracellular matrix proteins in different MC groups. MCs from PD effluent with the nonepithelioid phenotype show clear upregulation of collagen I and IV and fibronectin.

ters as VEGF levels in sera and supernatants. Comparisons between data groups were performed by using the nonparametric Mann-Whitney rank-sum *U* test, Spearman regression analysis, chi-square, and 2-tail Fisher exact test. *P* less than 0.05 is considered statistically significant. We used the statistical program SPSS, version 11.5 (SSPS Inc, Chicago, IL).

RESULTS

Upregulation of VEGF Expression in Transdifferentiated MCs

In this study, we grouped transitional and fibroblast-like MCs into a single category, hereafter referred to as nonepithelioid MCs (Fig 1A). As shown in Fig 1B, nonepithelioid MCs produced greater amounts of extracellular matrix components than epithelial-like MCs from PD effluent and omentum-derived MCs, reinforcing the concept of EMT of nonepithelioid cells. In addition, effluent MCs showed a progressive increase in VEGF production ex vivo as transdifferentiation proceeded, with VEGF levels maximum in nonepithelioid MC culture supernatants (Fig 2A). Transforming growth factor β plus interleukin 1 treatment of omentum-derived MCs to induce transdifferentiation in vitro resulted in increased VEG production (Fig 2B).

Serum VEGF Concentrations in PD Patients

When PD patients were subdivided into 2 groups according to the phenotype of MCs in their effluent, significantly greater serum VEGF levels were observed in patients who drained nonepithelioid MCs (Fig 3). Furthermore, there was correlation ($r = 0.6$; $P < 0.01$) between VEGF production ex vivo by effluent MCs and serum VEGF levels of patients (data not shown). Circulating VEGF concentrations in PD patients with nonepithelioid MCs in effluent showed a tendency to be greater than in HD patients, although values did not reach statistical significance ($P = 0.078$). Conversely, values were significantly greater than in healthy controls ($P < 0.05$; Fig 3).

Analysis of baseline characteristics of the 37 PD patients included in the study and differences between subgroups according to morphological characteristics of effluent MCs showed important differences in serum and supernatant VEGF levels, Cr-MTC, urea-MTC, and ultrafiltration rate at 3.86% glucose (Table 1).

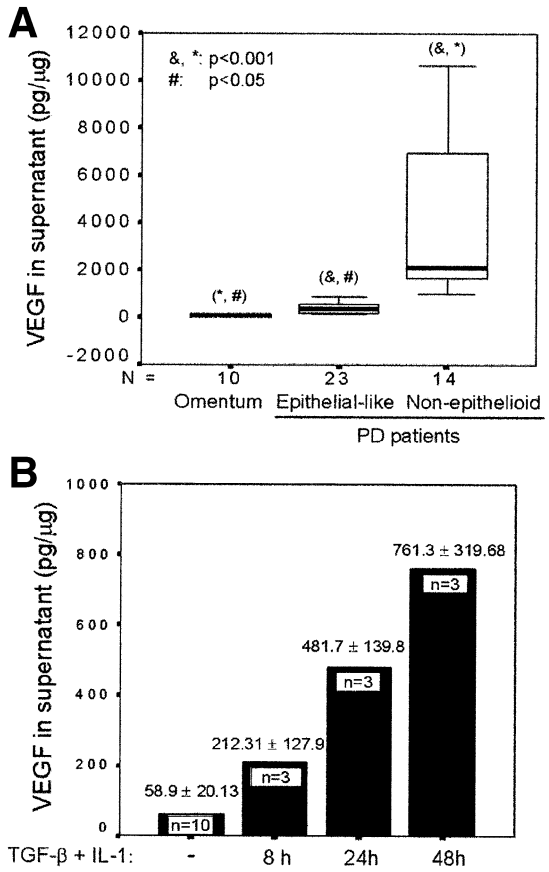

Fig 2. (A) VEGF production in supernatant (picograms per microgram) in MCs from omentum, epithelial-like, and nonepithelioid. Box plots represent 75% percentile, 25% percentile, median, maximum, and minimum values. Symbols show statistical differences between groups: omentum versus epithelial-like (mean, 58.9 ± 20.13 [SD] versus 377 ± 224.5; $P < 0.05$), omentum versus nonepithelioid (58.9 ± 20.13 versus 4,068 ± 3,521.3; $P < 0.001$), and epithelial-like versus nonepithelioid (377 ± 224.5 versus 4,068 ± 3,521.3; $P < 0.001$). (B) VEGF production in supernatant (picograms per microgram) in MCs from omentum treated with transforming growth factor β and interleukin 1 at various times.

Correlation Between Serum and Supernatant VEGF Concentrations and Peritoneal Transport Rate

Because increases in both Cr-MTC and urea-MTC are clinical markers of peritoneal permeability, which is related in turn to augmented blood vessel number, our data suggest that local VEGF production by MCs could have an important role in peritoneal membrane failure. Therefore, we analyzed the correlation between VEGF production in vivo and ex vivo and transport characteristics of PD patients. A significant positive linear correlation ($r = 0.35$; $P < 0.05$) between serum VEGF concentration and Cr-MTC was observed (Fig 4A). Most importantly, when Cr-MTC was related to VEGF production ex vivo by effluent-derived MCs, a strong logarithmic ($r = 0.8$; $P < 0.001$) correlation was obtained (Fig 4B). Thus, Cr-MTC correlated with both serum and supernatant VEGF concentrations (Fig 4C). Urea-MTC also correlated in a logarithmic manner ($r = 0.67$; $P < 0.01$) with supernatant VEGF concentration (data not shown). In addition, both MTCs showed a significant linear correlation ($r = 0.71$; $P < 0.01$; data not shown).

When PD patients were subdivided into 2 groups according to peritoneal transport characteristics (Cr-MTC < 11 mL/min [low and low-average transporters] and Cr-MTC > 11 mL/min [high and high-average transporters]), significantly greater serum ($P < 0.001$) and supernatant ($P < 0.001$) VEGF concentrations were observed in the last group (Fig 5). In addition, Cr-MTC was associated with effluent MC phenotype. No patient with epithelial-like MCs in their effluent showed a Cr-MTC greater than 11 mL/min, whereas 71% of patients (10 of 14 patients) with nonepithelioid MCs showed a Cr-MTC greater than 11 mL/min ($P < 0.001$; Table 2). It is important to note that the remaining 4 patients with nonepithelioid MCs and a Cr-MTC less than 11 mL/min showed a transitional, rather than fibroblast-like, phenotype in their effluent MCs.

Finally, significant positive linear correlations between months on PD therapy and serum VEGF concentrations ($r = 0.39$; $P < 0.05$) and between days of active peritoneal inflammation (peritonitis) and serum VEGF concentrations ($r = 0.41$; $P < 0.05$) were observed, supporting the notion that peritoneal damage contributes to local VEGF production.

VEGF Expression in Peritoneal Biopsy Specimens From PD Patients

To confirm in vivo that local overexpression of VEGF by nonepithelioid MCs was related to peritoneal transport failure, peritoneal biopsy specimens from 6 PD patients considered high transporters, 6 PD patients considered normal or low transporters, and 6 controls (nonrenal patients) were subjected to simple blind (the pa-

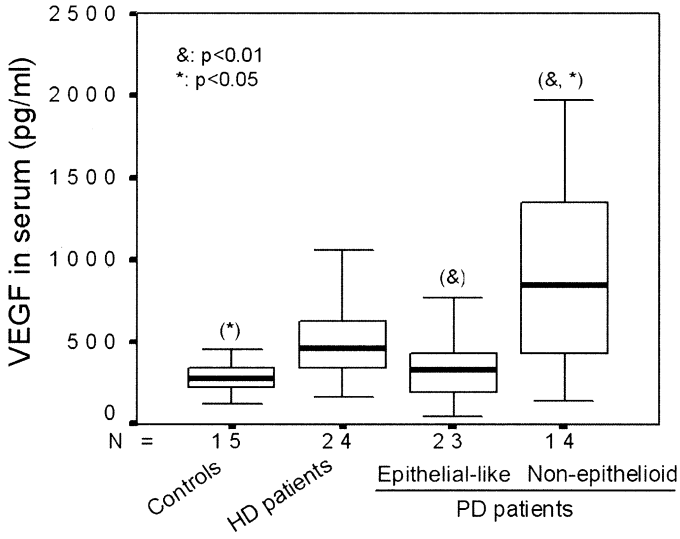

Fig 3. Serum VEGF (picograms per milliliter) levels in PD patients that drained epithelial-like or nonepithelioid MCs in effluent compared with HD patients and healthy controls. Box plots represent 75% percentile, 25% percentile, median, maximum, and minimum values. Symbols show statistical differences between groups: controls versus nonepithelioid (mean, 351 ± 220.6 [SD] versus 894.8 ± 624.3; $P < 0.05$) and epithelioid versus nonepithelioid (331.7 ± 190.4 versus 894.8 ± 624.3; $P < 0.01$). HD patients (456.5 ± 117.2) did not reach statistical differences with the other groups.

thologist) immunohistochemical analysis (Fig 6). Submesothelial fibroblasts from the control group showed no expression of VEGF, with its expression confined to the surface MC monolayer. Despite the low number of cases analyzed, in the high-transporter group, 65% of peritoneal samples (4 of 6 samples) showed clear VEGF expression in spindle-like cells embedded in the fibrotic stroma located mainly in the upper submesothelial area. Conversely, peritoneal biopsy specimens from normal or low transporters showed no or weak VEGF immunostaining in submesothelial fibroblast-like cells, and its expression was confined to deep endothelial cells. Interestingly, serial sections from the same peritoneal samples showed expression of cytokeratins, which overlapped with VEGF expression, in high transporters, whereas normal or low transporters did not show cytokeratin immunostaining in the submesothelial area (Fig 6). Given that the expression of cytokeratins is downregulated gradually during transdifferentiation of MCs, it can be speculated that fibroblast-like cells positive for this marker represent only a portion of the entire population of fibroblastic cells that derive from the mesothelium.[5,6]

DISCUSSION

Peritoneal fibrosis is one of the most common structural changes observed in patients undergoing PD. Degree of fibrosis correlates with time on PD therapy and episodes of peritonitis or hemoperitoneum. For a long time, fibrosis has been considered the main cause of the progressive functional decline in the peritoneum and ultrafiltration failure. In parallel with fibrosis, the peritoneum also shows a progressive increase in capillary number and vasculopathy in response to PD. In this context, recent reports evidenced that enhancement of peritoneal vasculature and vessel permeability is responsible for increased solute transport across the peritoneal membrane and ultrafiltration failure.[3,10,22]

Pathophysiological characteristics of peritoneal functional impairment during long-term PD therapy have remained elusive for a long time. Previous studies showed that MCs from omentum have the capacity to produce VEGF in vitro in response to a variety of stimuli, such as glucose degradation products,[14] advanced glycation end products,[16] transforming growth factor β,[17] and PD fluids.[18] Furthermore, effluent-derived MCs produce spontaneously different levels of VEGF ex vivo, but the reason for these different VEGF production abilities were not established.[23] Results presented in this study clearly show for the first time that the mechanism underlying VEGF upregulation in MCs is EMT of these cells, which is induced by PD. In addition, we also show that patients with nonepithelioid MCs in their effluent show greater circulating VEGF concentrations than those with epithelial-like MCs in effluent. Interestingly, there is a positive correlation between spontaneous VEGF synthesis ex vivo by effluent MCs and serum VEGF concentration, suggesting that MCs

Table 1. Baseline Characteristics and Differences in PD Patients With Different Phenotypes of MCs Cultured From PD Effluent

Parameter	Studied Population (N = 37)	MC Phenotype		P
		Epithelial-Like (n = 23)	Nonepithelioid (n = 14)	
Age (y)	62 ± 14.5	60.6 ± 13.8	64.3 ± 16	NS
Time on PD (mo)	12.8 ± 15.4	10.52 ± 15	16.6 ± 15.8	NS
EPO (U/kg/wk)	94.6 ± 94.8	83.9 ± 99.1	112.3 ± 88.1	NS
Peritoneal glucose load (kg)	54.9 ± 34.2	45.2 ± 32.6	64.5 ± 34.8	0.06 (NS)
Urea-MTC (mL/min)	19.8 ± 4.9	17.8 ± 2.8	23.1 ± 5.8	<0.01
Cr-MTC (mL/min)	9.3 ± 2.7	7.94 ± 1.65	11.46 ± 2.6	<0.001
Ultrafiltration 3.86% (mL)*	640 ± 242.3	786.2 ± 117†	508.5 ± 184.1	<0.05
Creatinine clearance (mL/s)	0.08 ± 0.06	0.09 ± 0.06	0.07 ± 0.05	NS
VEGF serum (pg/mL)	375 (42-1,972)	331 (42-876)	847 (139-1,972)	<0.01
VEGF supernatant (pg/μg)	646 (107-10,630)	360 (107-1,100)	2,103 (972-10,630)	<0.001

NOTE. Values expressed as mean ± SD or median (range) for non–normally distributed parameters (serum and supernatant VEGF).

Abbreviations: EPO, recombinant human erythropoietin; NS, not significant.

*Peritoneal exchange with 3.86% glucose (214.3 mmol/L) during 4 hours.

†Ultrafiltration of 1 patient from the epithelial-like group was not available; therefore, in this case, n = 22.

are an important source of VEGF in PD patients and that drained MCs retain their capacity to produce different VEGF levels despite being cultured in homogeneous conditions, at least during the first passage. Our results also indicate that EMT of MCs is involved not only in peritoneal fibrosis, but also in triggering and maintaining peritoneal angiogenesis. Therefore, results obtained with drained MCs in terms of VEGF production ex vivo are keys for understanding the behavior of MCs in the peritoneum.

Peritoneal membrane function is determined by ultrafiltration and small-solute MTC. In agreement with previous results,[12] we found that serum VEGF concentration correlates with Cr-MTC. In this study, we did not measure VEGF levels in dialysate; however, some previous reports,[12-14,24] but not others,[25] also described a correlation between VEGF concentration in PD effluent and peritoneal membrane failure. These discrepancies and the relatively weak associations between serum VEGF level and peritoneal transport rate and between dialysate VEGF level and peritoneal transport rate could be caused by multifactorial influences for VEGF production in vivo; ie, cardiovascular diseases in uremic patients.[26] These multiple factors are no longer present in MC cultures. Therefore, one important finding of this work is that VEGF production ex vivo by effluent MCs shows a stronger correlation with peritoneal transport rate than serum or effluent VEGF concentrations.

Rodrigues et al[27] showed a correlation between MC mass, measured as effluent cancer antigen 125 (CA125), and effluent VEGF level in patients on PD therapy for a few months. In addition, both levels (CA125 and VEGF) correlated with peritoneal transport rate. Conversely, the relationship between CA125 level and both effluent VEGF level and peritoneal transport rate disappeared during long-term PD therapy, probably because of a decline in MC mass. However, these patients maintained the correlation between effluent VEGF level and peritoneal transport rate, and the investigators suggested a non-MC source for VEGF production in late PD. In this study, we clearly show that MCs that have undergone EMT produce a greater quantity of VEGF. These transdifferentiated MCs are located, at least in part, embedded in the submesothelial area because of their increased migratory capacity.[5,6] In this context, our immunohistochemical analysis of peritoneal biopsy specimens from PD patients showed upregulation of VEGF in stromal spindle-like cells in high transporters, but not low transporters, which derived from the mesothelium. Therefore, although MC mass (CA125) declines during long-term PD therapy, MCs that remain in the submesothelial peritoneum, in their mesenchymal stage,

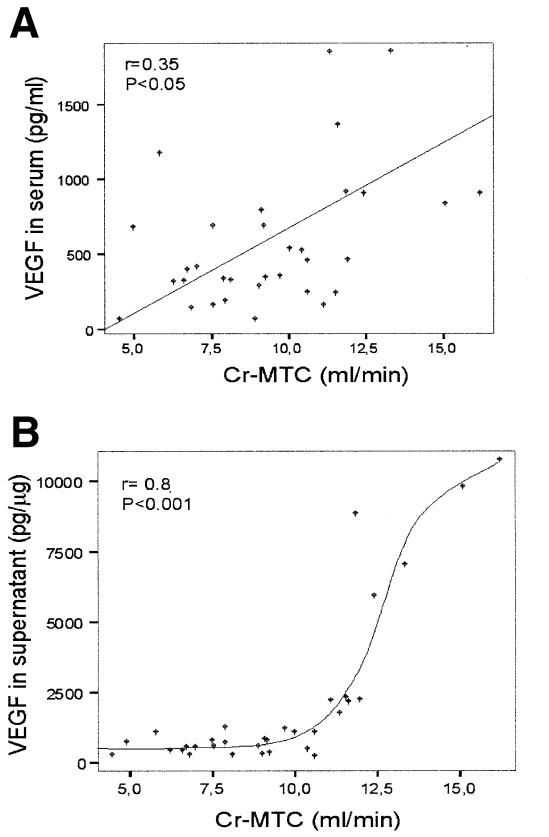

Fig 4. (A) Linear correlation between VEGF levels in serum and Cr-MTC (r = 0.35; P < 0.05) in the PD patient group. (B) Logarithmic correlation between VEGF level in supernatant and Cr-MTC (r = 0.8; P < 0.001). (C) Graphic in 3 dimensions representing the relationship between supernatant, serum VEGF level, and Cr-MTC. Black dots, MCs from PD effluent with epithelial-like phenotype (n = 23); white dots, nonepithelioid phenotype (n = 14).

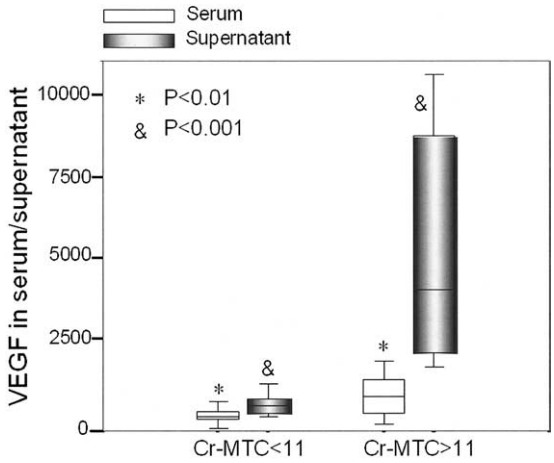

Fig 5. Differences in serum and supernatant VEGF levels in patients with a Cr-MTC less than 11 mL/min (low and low-average transporters) and Cr-MTC greater than 11 mL/min (high and high-average transporters). Serum VEGF values in picograms per milliliter; supernatant VEGF, picograms per microgram. Box plots represent 75% percentile, 25% percentile, median, maximum, and minimum values. Symbols show statistical differences between groups: serum VEGF in patients with a Cr-MTC less than 11 mL/min versus Cr-MTC greater than 11 mL/min (mean, 402 ± 361.5 [SD] versus 928.8 ± 596.7; P < 0.01), supernatant VEGF levels in patients with a Cr-MTC less than 11 mL/min versus Cr-MTC greater than 11 mL/min (506 ± 404 versus 5,196.1 ± 3,591.8; P < 0.001).

also may be responsible for peritoneal transport abnormalities through VEGF overexpression. Although CA125 classically has been used as an index of MC mass in PD patients, recently, the value of CA125 has been questioned because such factors as age and glucose concentration may affect its expression.[28] Thus, use of CA125 as a marker of MC preservation has a limited value. Nowadays, there is no information on

Table 2. Distribution of MC Phenotypes According to Peritoneal Transport Rate

	MC Phenotype		
	Epithelial-Like	Nonepithelioid	Total
Cr-MTC < 11 mL/min	23	4	27
Cr-MTC < 11 mL/min	0	10	10
Total	23	14	37

NOTE. Statistically significant differences in distribution of epithelial-like and nonepithelioid phenotypes in groups of low, low-average (Cr-MTC < 11 mL/min), and high, high-average (Cr-MTC > 11 mL/min) peritoneal transporters (2-tail Fisher exact test, P < 0.001).

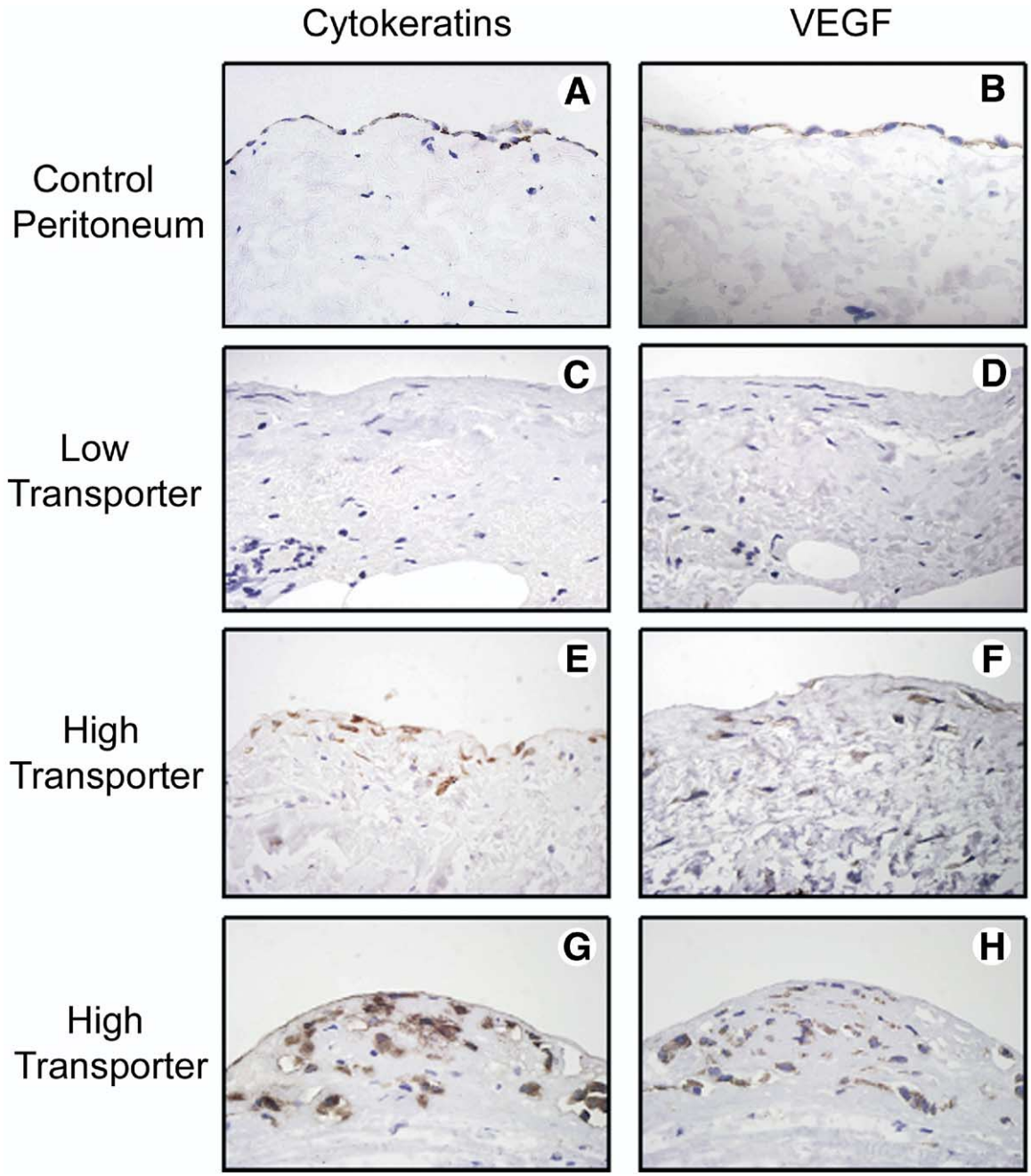

Fig 6. Immunoexpression of (A, C, E, G) cytokeratins and (B, D, F, H) VEGF in representative peritoneal biopsy samples from (A, B) controls and (C, D) low-normal transporter and (E-H) high-transporter PD patients.

CA125 behavior during EMT of MCs; therefore, no conclusion can be inferred from analysis of the association between CA125 and VEGF levels in effluent from long-term PD patients, in which EMT of MCs is massive.

The observation that transdifferentiated MCs invade the submesothelial stroma[5,6] implies that one of the main sources of VEGF in the injured peritoneum is entrapped into the extracellular matrix. Thus, it can be hypothesized that MC-

derived VEGF exerts its effects in a paracrine manner, inducing local angiogenesis and vascular permeability, and only a limited and variable proportion of this VEGF escapes to the effluent and circulation compartments. This could be an additional explanation for the divergent strength of correlations between peritoneal transport rate and VEGF levels produced in vivo (serum and effluent) or ex vivo. Follow-up of VEGF produced ex vivo by effluent MCs may serve as a marker to evaluate the evolution of peritoneal membrane functionality in PD patients.

In this study, we were not able to establish differences between patients using different PD solutions in VEGF production in vivo and ex vivo because of the low number in each group. However, it would be worth comparing the classic and new PD solutions in terms of biocompatibility by using ex vivo VEGF level as a peritoneal function diagnosis marker in longitudinal follow-up.

Data that point to EMT of MCs as a key process in the initiation of peritoneal fibrosis and angiogenesis open new insights for therapeutic intervention. Treatments may be designed toward either direct prevention of EMT of MCs or its deleterious effects, such as extracellular matrix synthesis and/or VEGF production. In this context, it was shown that human growth factor, bone morphogenic protein-7, and inhibitors of the integrin linked-kinase and RhoA-Rho-kinase pathways are able to inhibit and reverse both tissue fibrosis and EMT of tubular epithelial cells in animal models of renal fibrosis.[29] It is conceivable that intervention in either local VEGF synthesis[30] or VEGF activity[31] also might prevent peritoneal neovascularization and ultrafiltration failure. Although VEGF is an important proangiogenic factor, other molecules, such as nitric oxide and angiopoietins, also are involved in endothelial cell proliferation, vascular permeability, and vessel stabilization. Thus, additional studies to analyze the behavior of these molecules during EMT of MCs are required.

In conclusion, our findings suggest that the new fibroblast-like cells that arise from local conversion of MCs by EMT during the repair responses of peritoneal tissue may retain a permanent mesenchymal state as long as initiating stimuli persist and may contribute not only to PD-induced fibrosis, but also to angiogenesis and vascular permeability of the peritoneum through VEGF upregulation.

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6.1.4.2.- "La transición epitelio-mesenquimal de las células mesoteliales es un evento precoz durante la diálisis peritoneal y está asociada con alto transporte peritoneal".

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Kidney International Supplement 2008; 73: S26-S33

Este trabajo responde al objetivo 2

Este estudio ha recibido el Premio de la Fundación Renal Iñigo Álvarez de Toledo (FRIAT) al mejor trabajo de investigación clínica en el año 2009 (concedido a Gloria del Peso Gilsanz)

La transición epitelio-mesenquimal de las células mesoteliales es un fenómeno precoz durante el tratamiento con diálisis peritoneal y está asociada con el alto transporte peritoneal.

El fallo de UF es una consecuencia del tratamiento a largo plazo de pacientes con DP, y se ha relacionado con la presencia en peritoneo de lesiones de fibrosis, angiogénesis y vasculopatía en pacientes con más de 3 años en la técnica. La TEM de la célula mesotelial se ha propuesto como un elemento clave en el desarrollo de fibrosis y deterioro funcional a nivel peritoneal.

Objetivo: Evaluar los cambios morfológicos que se producen durante los primeros meses del tratamiento con la DP y establecer su posible correlación con los cambios en los parámetros de función peritoneal.

Métodos: Se analizaron las biopsias de peritoneo parietal de 35 pacientes estables en DP con menos de dos años en la técnica (tiempo medio en DP de 13.8±6.6 meses). Se excluyeron los pacientes con problemas funcionales peritoneales o con datos de inflamación local o sistémica. Su edad media era de 45.37± 14.5 años. Se establecieron como controles un grupo de diez biopsias peritoneales obtenidas de pacientes sin patología renal ni problemas abdominales.

Resultados: El 74% de las muestras peritoneales presentaba pérdida parcial o total de la capa mesotelial, 46% signos de fibrosis submesotelial (espesor submesotelial >150 μm) y en un 17% de los casos se evidenció la presencia in situ de TEM (células fibroblásticas submesoteliales con expresión de citoqueratina). Todos los pacientes con TEM tenían miofibroblastos a nivel submesotelial, mientras que solamente en el 36% de pacientes sin TEM se

identificaron. En un 17% de las biopsias se evidenciaron lesiones de VH. El número de vasos fue similar en todos los periodos de tiempo en DP.

Los pacientes pertenecientes al cuartil más alto de transporte peritoneal de creatinina (MTC-Cr>11.8 ml/min) mostraron mayor prevalencia de TEM que el resto de pacientes (p=0.016), pero un número similar de vasos peritoneales. Además, en el análisis multivariante, el cuartel superior de MTC-Cr permaneció como un factor predictor independente de presencia de TEM (odds ratio 12.4; intervalo de confianza: 1.6–92; p=0.013), incluso tras el ajuste para la presencia de fibrosis (p=0.018).

Conclusiones: La TEM de las células mesoteliales es una alteración morfológica peritoneal frecuente en pacientes con menos de dos años en tratamiento con DP. El alto transporte peritoneal de pequeños solutos se asocia con la presencia de TEM, pero no con un mayor número de vasos a nivel peritoneal.

Epithelial-to-mesenchymal transition of mesothelial cells is an early event during peritoneal dialysis and is associated with high peritoneal transport

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Ultrafiltration (UF) failure is a consequence of long-term peritoneal dialysis (PD). Fibrosis, angiogenesis, and vasculopathy are causes of this functional disorder after 3–8 years on PD. Epithelial-to-mesenchymal transition (EMT) of mesothelial cell (MC) is a key process leading to peritoneal fibrosis with functional deterioration. Our purpose was to study the peritoneal anatomical changes during the first months on PD, and to correlate them with peritoneal functional parameters. We studied 35 stable PD patients for up to 2 years on PD, with a mean age of 45.3 ± 14.5 years. Seventy-four percent of patients presented loss of the mesothelial layer, 46% fibrosis (> 150 μm) and 17% *in situ* evidence of EMT (submesothelial cytokeratin staining), which increased over time. All patients with EMT showed myofibroblasts, while only 36% of patients without EMT had myofibroblasts. The number of peritoneal vessels did not vary when we compared different times on PD. Vasculopathy was present in 17% of the samples. Functional studies were used to define the peritoneal transport status. Patients in the highest quartile of mass transfer area coefficient of creatinine (Cr-MTAC) (> 11.8 ml min^{-1}) showed significantly higher EMT prevalence ($P = 0.016$) but similar number of peritoneal vessels. In the multivariate analysis, the highest quartile of Cr-MTAC remained as an independent factor predicting the presence of EMT (odds ratio 12.4; confidence interval: 1.6–92; $P = 0.013$) after adjusting for fibrosis ($P = 0.018$). We concluded that, during the first 2 PD years, EMT of MCs is a frequent morphological change in the peritoneal membrane. High solute transport status is associated with its presence but not with increased number of peritoneal vessels.

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KEYWORDS: epithelial-to-mesenchymal transition; mesothelial cell; peritoneal high transport; submesothelial fibrosis; peritoneal biopsy

Chronic peritoneal dialysis (PD) for end-stage renal disease treatment has been used for more than 30 years.[1] Nowadays, the expansion of PD continues to be limited by the membrane incapacity to perform diffusive and/or convective transport over the long term.[2] Water, sodium, and small solute transports can all be affected by this limitation.[3–7] The worst functional consequence is UF failure, which results in extracellular volume overload, increased cardiovascular risk, and the restriction for technique continuity.[8–11] Functional deterioration of the peritoneum is related to the damage induced by components of PD fluids, most likely pH, glucose, and glucose degradation products. Therefore, during the last decade many efforts have been made to improve the biocompatibility of fluids. Such an improvement of biocompatibility is expected to result in less peritoneal damage. Recently, important contributions from cell biology and histopathology studies have helped us to understand the pathophysiology of the peritoneal membrane response.[12–18] Epithelial-to-mesenchymal transition (EMT) of MC has been identified as a key process leading to peritoneal fibrosis with functional deterioration.[19,20] Concerning human histopathology studies, it must be remarked that most peritoneal biopsy studies have been based on PD patients with long-term treatment and peritoneal functional problems, specifically UF failure.[21–24] As a consequence, advanced morphopathological changes such as fibrosis, angiogenesis, and vasculopathy are probably overrepresented. These lesions are the main cause of functional disorder after 3–8 years on PD.[23,25] Obtaining peritoneal biopsies from short- to medium-term PD patients with no functional anomalies is not easy. Except for renal transplantation, there are few other opportunities to have access to a noninjured peritoneal membrane. The understanding of pathologic

processes leading to advanced peritoneal anatomical–functional disorders requires recognition of the earlier key points. Knowledge of premature peritoneal changes might reveal information sufficient to interpret the primary response to PD. The main objective of the present study was to examine such an initial phase of PD treatment. For this purpose, we have explored the peritoneal anatomical changes appearing during the first months on PD, and correlated these findings with peritoneal functional parameters determined in the same period.

Table 1 | Peritoneal functional data of the whole series

Patients	35
Peritonitis episodes	7
Days of peritonitis	2.7±2
Urea-MTAC (ml min^{-1})	19.7±6
Cr-MTAC (ml min^{-1})	8.7±4.6
UF (ml per 4 h)	871±283
D/P creatinine	0.7±0.09

D/P, dialysate/plasma; Cr-MTAC, mass transfer area coefficient of creatinine; Urea-MTAC, mass transfer area coefficient of urea; UF, ultrafiltration.

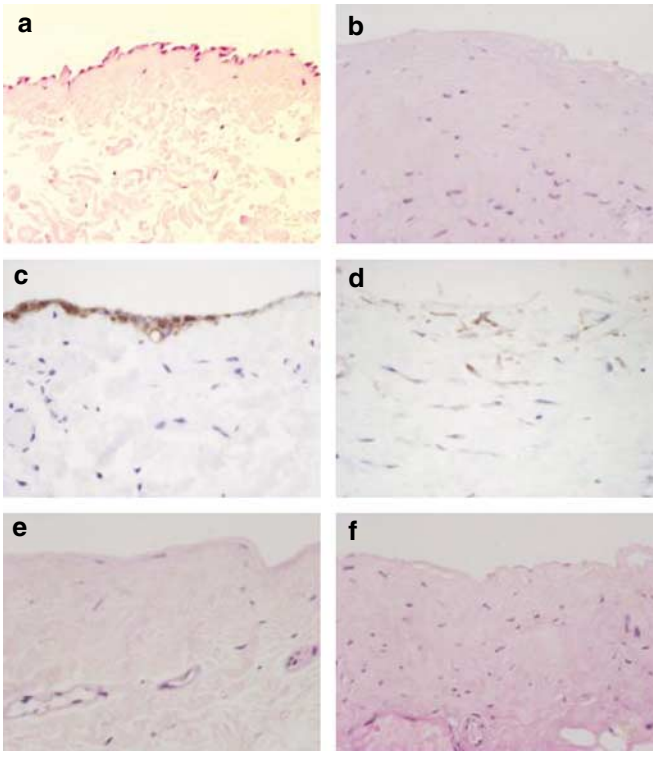

Figure 1 | Biopsy findings in normal and high transport patients. (**a** and **c**) Biopsy samples from patients with normal transport that show no relevant fibrosis (**a**, hematoxylin–eosin, original magnification ×100), and absence of cytokeratin + submesothelial fibroblasts (**c**, immunoperoxidase, original magnification ×150). (**b** and **d**) Samples from high transport patients and show fibrosis (**b**, hematoxylin–eosin, original magnification ×100) and cytokeratin + submesothelial fibroblasts (**d**, immunoperoxidase, original magnification ×150). (**e** and **f**) Samples from normal and high transport patients show a similar number of vessels (three and two, respectively, hematoxylin–eosin, original magnification ×100).

RESULTS

Analysis of data from the complete series of biopsies

Since the low number of patients at first year ($n=15$) does not permit a deeper analysis, we performed the analysis of the overall group (35 patients) as a whole (Table 1).

Histopathological findings

Mesothelial layer. Seventy-four percent of patients presented partial or total loss of the mesothelial layer. Forty percent of them showed no mesothelium at all.

Submesothelial zone: epithelial-to-mesenchymal transition features. Sixteen patients (46%) showed some degree of submesothelial thickness (>150 μm) or fibrosis (the terms submesothelial thickness and fibrosis are used in this paper indistinctly) (Figure 1). Patients with submesothelial thickening had similar mean time on PD than patients without fibrosis (13.9±6.4 vs 13.8±7 months, $P=0.97$). The prevalence of submesothelial fibrosis did not vary during time on PD when we analyzed the four semesters (Figure 2). *In situ* evidence of EMT was present in six patients (17%). Mean time on PD was not statistically significantly different between patients with or without EMT (15±8.6 vs 13.6±6.3 months, $P=0.56$). There was a trend to a higher prevalence of EMT in the fourth semester on PD, but the low number of patients does not permit the statistical analysis to be performed (Figure 3). Submesothelial thickness was not associated with the presence of EMT: 83% patients with EMT had fibrosis, but 38% patients without EMT also showed fibrosis ($P=0.07$).

Forty-seven percent of the biopsies showed myofibroblasts. All patients with EMT showed myofibroblasts, while only 36% of patients without EMT had myofibroblasts ($P=0.006$). One-third of patients with myofibroblasts (α-smooth-muscle actin +) also showed EMT.

Vessel density. The number of peritoneal vessels did not vary when we compared different times on PD (Figure 4).

Vasculopathy. Mild degree of vasculopathy was present in six patients (17% of the samples). Moderate degree was present only in 3% of patients, with no cases with severe vasculopathy. Patients with and without vasculopathy showed similar mean time on PD (13.6±4.6 vs 13.9±7 months, $P=0.93$). The prevalence of vasculopathy was similar in different semesters of treatment (Figure 5). There

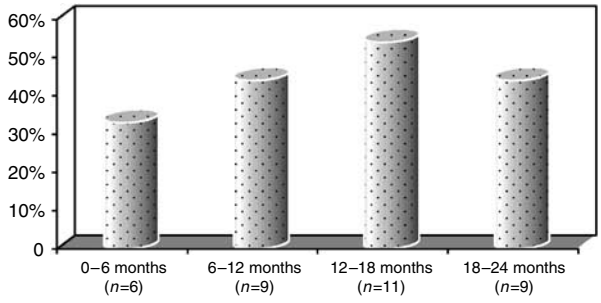

Figure 2 | Prevalence of submesothelial fibrosis according to different semesters. The prevalence of submesothelial fibrosis was similar in the different semesters.

was no association between vasculopathy and fibrosis (83% patients with vasculopathy had fibrosis and 38% patients without vasculopathy had fibrosis; $P = 0.073$) or EMT (33% of patients with vasculopathy had EMT and 14% patients without vasculopathy had EMT; $P = 0.26$). Fifty percent of patients with vasculopathy had myofibroblasts, and 46% patients without vasculopathy had myofibroblasts; $P = 1.00$. Table 2 summarizes the prevalence of different peritoneal lesions along the time observed.

Morphofunctional correlations

The different peritoneal lesions were related to the peritoneal functional parameters divided into quartiles. Patients in the highest quartile of mass transfer area coefficient of creatinine (Cr-MTAC) and the median value of UF were considered the reference. Table 3 shows the prevalence of peritoneal lesions in the four quartiles of Cr-MTAC and UF values.

Small solute peritoneal transport. Patients in the highest quartile of Cr-MTAC ($>11.8\,\text{ml}\,\text{min}^{-1}$) showed significantly higher prevalence of EMT ($P = 0.016$) (Figure 6) and similar presence of myofibroblasts, fibrosis, and vasculopathy ($P = 1.00$, NS) than the other quartiles. However, all showed a similar prevalence of fibrosis (first quartile, 30%; second quartile, 50%; third quartile, 56%; and fourth quartile, 50%), as well as a similar number of peritoneal vessels (first quartile: 3 ± 1 vessels per field, $n = 8$; second quartile: 4.7 ± 2 vessels per field, $n = 2$; third quartile: 4.4 ± 1 vessels per field, $n = 2$; and fourth quartile: 4.3 ± 2 vessels per field, $n = 6$) (NS).

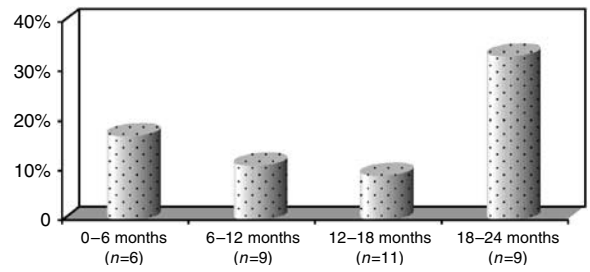

Figure 3 | Prevalence of EMT according to different semesters. We found a trend to a higher prevalence of EMT in the fourth semester on PD, around 30%, when compared with the remaining, around 10–15%. The low number of patients does not permit to perform the statistical analysis between the four semesters.

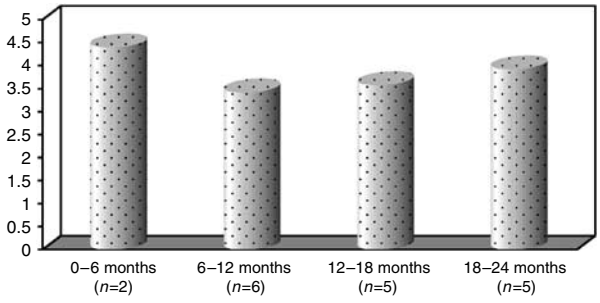

Figure 4 | Vascular density according to different semesters. The number of peritoneal vessels was similar in different times on PD, with no change along time on PD.

We found no relation between the presence of EMT and previous peritonitis, since one of the six patients with EMT (17%) had a previous episode of peritonitis in contrast to 6 of the 29 patients (21%) without EMT ($P = 0.82$). Days of peritonitis did not influence these results either (2 days in EMT group vs 2.8 days in non-EMT, NS)

Ultrafiltration capacity. No correlation was found between the peritoneal lesions evaluated and UF capacity. When we compared patients over and under the median value of UF (820 ml per 4 h), we found a trend to a higher prevalence of fibrosis in patients with lower UF capacity (over the median: 29.4% and under the median: 61.1%) ($P = 0.06$). No statistical differences were found in the prevalence of EMT ($>$ median: 5.9%, $<$ median: 27.8%) (NS), myofibroblast presence ($>$ median: 44%, $<$ median: 50%) (NS), vasculopathy ($>$ median: 18%, $<$ median: 17%) (NS), and number of vessels ($>$ median: 3.45 ± 1.2 vessels per field, $n = 8$; $<$ median: 4.12 ± 1.8, $n = 10$) (NS).

Logistic regression analysis. Table 4 shows the univariate analysis data (unadjusted odds ratio) for the presence of EMT in peritoneal biopsies. In the multivariate analysis, the highest quartile of Cr-MTAC ($>11.8\,\text{ml}\,\text{min}^{-1}$) remained as an independent factor predicting the presence of EMT (odds ratio 12.4; confidence interval: 1.6–92; $P = 0.013$) after adjusting for fibrosis ($P = 0.018$). None of the variables included in our study significantly predicted the presence of submesothelial thickness or vasculopathy in peritoneal biopsies.

Analysis of data from biopsies taken during the first year

The six patients with biopsies obtained during the first 6 months on PD with no peritonitis showed two cases (33%)

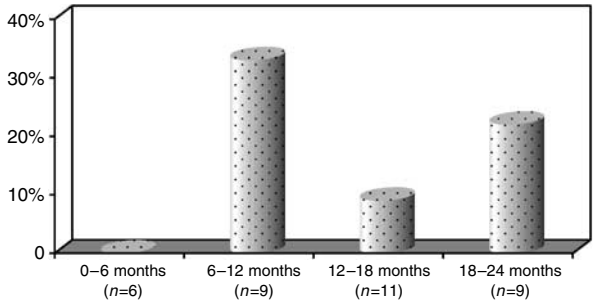

Figure 5 | Prevalence of vasculopathy according to different semesters. The prevalence of vasculopathy was similar in different semesters of treatment. None of the cases with vasculopathy was found in the first 6 months of treatment, and variable prevalence was present in the remaining times on PD.

Table 2 | Prevalence of different peritoneal lesions along time

	First semester	Second semester	Third semester	Fourth semester
Submesothelial fibrosis	33%	44%	54%	44%
EMT	17%	11%	9%	33%
Vasculopathy	0	33%	9%	22%
Number of peritoneal vessels per field	4.4	3.7	3.6	3.9

EMT, epithelial-to-mesenchymal transition.

Table 3 | Prevalence of peritoneal-specific lesions in different quartiles of solute and water transport for patients studied during the first year on PD

	Cr-MTAC				UF capacity			
	First quartile	Second quartile	Third quartile	Fourth quartile	First quartile	Second quartile	Third quartile	Fourth quartile
Submesothelial fibrosis	25%	50%	25%	67%	0	100%	25%	33%
EMT	0	0	25%	33%	25%	0	0	33%
Vasculopathy	25%	25%	0	33%	0	25%	25%	33%
Number of peritoneal vessels per field	3.6	3.6	3.6	4	3.3	4	3.5	5.5

Cr-MTAC, mass transfer area coefficient of creatinine; EMT, epithelial-to-mesenchymal transition; PD, peritoneal dialysis; UF, ultrafiltration.

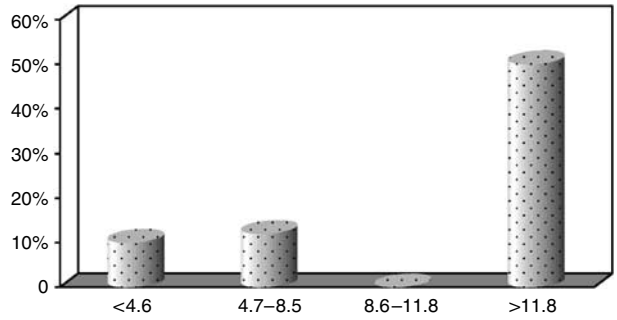

Figure 6 | Prevalence of EMT according to Cr-MTAC quartiles. We observed that patients in the highest quartile of Cr-MTAC ($>11.8\,ml\,min^{-1}$) showed higher prevalence of EMT than other quartiles of Cr-MTAC. When we compared patients in the higher quartile (Cr-MTAC $>11.8\,ml\,min^{-1}$) with patients with Cr-MTAC lower than $11.8\,ml\,min^{-1}$, significant differences were found ($P=0.016$).

Table 4 | Univariate analysis

	OR (95% confidence interval)	*P*
Age (years)	1.05 (0.98–1.12)	0.10
Time on PD (months)	1.03 (0.90–1.18)	0.63
β-blockers use (yes/no)	0.81 (0.12–5.23)	0.83
ACEI use (yes/no)	3.05 (0.31–29.7)	0.33
ARA-II use (yes/no)	0.62 (0.06–6.32)	0.69
Previous peritonitis episodes (yes/no)	0.76 (0.75–7.86)	0.82
Accumulated days of peritonitis	0.72 (0.13–3.82)	0.70
Cr-MTAC $>11.8\,ml\,min^{-1}$ (fourth quartile)	12.49 (1.69–92.23)	0.01
Median UF (ml per 4 h)	0.16 (0.01–1.57)	0.11
Fibrosis (yes/no)	8.17 (0.84–79.36)	0.07
Vasculopathy (yes/no)	3.12 (0.42–23.06)	0.26
Number of vessels per field	1.43 (0.68–2.98)	0.33

ACEI, angiotensin converting enzyme inhibitor; ARA-II, angiotensin II receptor antagonist; EMT, epithelial-to-mesenchymal transition; Cr-MTAC, mass transfer area coefficient of creatinine; OR, odds ratio; PD, peritoneal dialysis; UF, ultrafiltration. Unadjusted OR for the presence of EMT in peritoneal biopsies.

with submesothelial fibrosis, one (16%) with EMT tissue data, none with vasculopathy and a normal number of vessels (mean 4.45 ± 1.2 vessels per field). In these patients, Cr-MTAC ranged from 8.7 to $16.6\,ml\,min^{-1}$, and UF capacity ranged from 500 to 1800 ml per 4-h 3.86% glucose dwell time. Since this group consists of few patients, we examined the data from the 15 patients studied within the first year. The inclusion of two patients who have suffered peritonitis did not demonstrate significant differences when compared to the other 13. The quartile distribution of Cr-MTAC was marked by the following values: 6.26, 11.3, and $13\,ml\,min^{-1}$. The corresponding quartile distribution for UF was 610, 800, and 1050 ml per 4-h dwell time. One case showed data of UF failure (UF = 300 ml). The peritoneal biopsies showed six cases (40%) with submesothelial fibrosis, two (13%) with EMT tissue data, and three cases (20%) with vasculopathy and a normal number of vessels (mean 3.9 ± 1.2 vessels per field). When compared to data from the first semester patients, an increased prevalence of vasculopathy was present. The remaining variables were similar. One of the two patients who had experienced peritonitis, showed fibrosis and vasculopathy.

DISCUSSION

This study, based on peritoneal biopsies performed during the first 2 years on PD, showed that EMT of MCs is a frequent peritoneal morphological change more common in those patients with higher solute transport status, and that such higher transport status was not associated with an increase in the number of vessels. A new interpretation of the mechanisms associated with fast peritoneal solute transport at early PD stages arises from these data.

Mesothelial-to-mesenchymal transition is defined, *in vivo*, by the presence of fibroblastic-like cells located in the submesothelium that express mesothelial markers such as cytokeratins. During their conversion into myofibroblasts, MCs gradually lose their location, morphology, and immunophenotype. What we detect using immunohistochemistry against cytokeratin is a subset of myofibroblasts or transitional cellular forms that still retain cytokeratin expression, reflecting their mesothelial origin. When final conversion has occurred, myofibroblasts will have lost the expression of cytokeratin and other mesothelial markers. Therefore, although specific, this detection method has low sensitivity since only a portion of transdifferentiated MCs will be detected.

Mesothelial cell detachment was seen in 74% of the peritoneal biopsies. Almost half of the tissue samples showed

some degree of submesothelial thickening. The two main features of EMT, that is cytokeratin and α-smooth-muscle actin+ submesothelial fibroblasts, were present in 17 and 47% of biopsies, respectively. All biopsies with cytokeratin+ fibroblasts also showed myofibroblasts, while 36% of patients with no cytokeratin+ fibroblasts had evidence of myofibroblastic differentiation ($P = 0.006$). One-third of patients with myofibroblasts (α-smooth-muscle actin+) also showed EMT.

A mild-to-moderate grade of vasculopathy was present in 17% of the series, and its association with submesothelial thickening and myofibroblast was sporadic. Longer time on PD was associated with submesothelial fibrosis only when EMT was also present. Analyzing the EMT findings over time, a remarkable higher prevalence of these findings at the fourth semester was demonstrated. It can be assumed that time on PD is a risk factor for submesothelial thickening associated with EMT after the first year. In fact, our previous findings in biopsies taken at longer PD periods[26] confirmed a progressive increase in EMT incidence (up to 48%). Myofibroblast presence in biopsies was very erratic over the time examined. Although they are characteristics of EMT process, fibroblasts from other origins must be participating in the process as well.[27–29]

Vasculopathy was absent during the first semester and present to a mild degree in the other periods examined. Its presence at these early stages reinforces the role of this lesion in the peritoneal changes secondary to PD. Vessel density (to be discussed also in transport discussion) remained stable over the periods examined. This observation suggests that vessel number remains similar during the first 2 years of a noncomplicated PD. In other words, the angiogenesis expected in later PD stages, has not yet started.

Data on solute transport and biopsy findings

One of the main concerns in peritoneal function is the status of the fast transporter. To avoid bias in the management of Cr-MTAC values in the study of its relationship with biopsy findings, we have divided this parameter into quartiles. In spite of the shortness of the series, we have had sufficient number of patients in each quartile to compare the biopsy findings. Figure 6 shows a remarkably different prevalence of EMT in fast transporters (the highest quartile of Cr-MTAC > $11.8\,ml\,min^{-1}$), approximately fivefold higher than in the other groups. The cut-off value of $11.8\,ml\,min^{-1}$ determines a true relationship with the presence of EMT in the biopsy, confirmed in the univariate and multivariate analyses. In consequence, we can firmly corroborate the association between higher solute transport and EMT in the biopsy. This association did not exist with submesothelial thickness *per se*. This seems to indicate qualitative differences in the composition of the thickened submesothelial zone. To confirm this transport–anatomical relationship, it was necessary to know whether or not the vascular density was consistent with the expectation that the higher the transport, the larger the number of vessels.[21,30]

Contrary to this paradigm, our patients have demonstrated that the sole presence of EMT and related fibrosis, with no increase in the number of capillaries, is sufficient to lead to a high transporter status. Other authors have found that angiogenesis is not necessarily associated with noncomplicated higher peritoneal transport at later PD stages (4–6 years).[31] Evidently, peritoneal lesions over PD time should be different in quality and quantity and probably are associated. The present data suggest that the early changes in response to PD are preceded by identifiable cell and extracellular matrix changes in the submesothelial compact zone. The increase in collagen and fibronectin,[26] prior to the increase of vessel number, can be sufficient. In fact, submesothelial thickening is the more constant finding detected in other peritoneal biopsy studies.[21,23] Animal models,[32] in which TGF-β transfection of MC is induced, reproduce the sequence of phenomena that seems to apparently occur in humans. These data have demonstrated that TGF-β transfection of rat MC causes peritoneal sclerosis, with previous development of angiogenesis, both processes anteceded by the EMT of MCs during the first 4 days after transfection.[33] The process in humans, as it does in the transfection model, should start by the EMT-induced change of MCs secondary to TGF-β effects and continue with submesothelial zone modifications. The EMT process starts by the loss of tight junctions (E-cadherin) by MC with their subsequent detachment into effluent and migration toward the submesothelial compact zone.[34–36] For this last purpose, cells develop migrating and invasive capabilities, in which vascular endothelial growth factor (VEGF) is involved.[37–39]

Our previous study has demonstrated the specific high capacity of VEGF production by transitional MCs in this situation.[40] Once cells are homed at submesothelial levels, they acquire capability of producing extracellular matrix components, collagen and fibronectin, and expand the surrounding submesothelial zone.[41] The presence of high tissue levels of VEGF also causes vasodilation of peritoneal capillaries. This phenomenon may be coresponsible for the association between VEGF and high transporter status.[42,43] Unfortunately, capillary dilation status cannot be quantified in biopsy due to simple technical reasons. To explain the mechanism through which the submesothelial EMT-related fibrosis influences peritoneal transport of solutes we have other considerations. The qualitative change of the peritoneal interstitium composition probably modifies the number and size of peritoneal pores. The potential absence of glycosaminoglycans, synthesized by normal MCs, and their replacement by collagen and fibronectin explain the great change. Interstitial changes have been sufficient to modify peritoneal transport in PD animal models with chronic inflammation.[44] We propose that what it is conceptually accepted for a normal membrane, the similarity of the transport in both directions, is modified by the structural change. In the new situation, a constellation of novel phenomena, including more and greater interstitial medium-size pores, relative absence of glycosaminoglycans, and some degree of vasodilation caused

by high presence of VEGF in tissue, might explain the rupture of the paradigm.

The UF capacity data from our patients complement this information, although in a less expressive manner. Patients with lower UF than the median demonstrated a greater prevalence of submesothelial fibrosis and EMT, both within the limit of statistical significance. The presence of myofibroblasts and vasculopathy was not related to UF capacity. Such capacity is the result of hydraulic permeability to water and the maintenance of osmotic gradient through the membrane. The peritoneal change observed in our patients may affect both conditions in different grades leading to an unpredictable consequence on the UF capacity. The manipulation of solute and water transport by intraperitoneal nonfractionated heparin suggests that the change of peritoneal membrane composition results in functional modifications.[45]

The limitations of our study include that it is cross-sectional and the shortness of the series that requires the contribution of other groups interested in the understanding of the peritoneal membrane change per stages, not only at the end of the process such as is generally considered the peritoneal biopsy registry in its current conception.

Prior mild peritonitis did not affect the results of this analysis, because they were not relevant for the presence of EMT (nonsignificant differences). A potential contribution in EMT of more aggressive peritonitis episodes may not be excluded. For future research in this field, progress in the knowledge of interstitium composition, collagen, fibronectin, and presence of glycosaminoglycans and their relationship to membrane transport are mandatory.

In conclusion, our data obtained from peritoneal biopsies performed during the first 2 years on PD demonstrate that the first morphological change in peritoneum that appears as a consequence of PD is submesothelial thickening partially caused by the EMT of MCs. This phenotype change is associated with an increase in peritoneal solute transport independent of the number of capillaries present in the tissue.

MATERIALS AND METHODS

Patients

Inclusion criteria.

- Stable patients up to 2 years on PD

Exclusion criteria.

- Systemic or local inflammatory condition
- Patients with PD treatment in a previous stage
- Peritoneal samples obtained from hernia sacs
- Patients with declared functional membrane failure

Groups of patients. The whole series was constituted of 35 patients (20 men and 15 women) with a mean age of 45.3 ± 14.5 years (range: 20–77). Twenty-six patients were on automated peritoneal dialysis and nine on continuous ambulatory peritoneal dialysis at the time of peritoneal biopsy. Three patients used low glucose degradation product dialysis solutions, one bicarbonate solution and the remaining patients were using lactate standard solution; 17 patients were using icodextrin. Ninety-seven percent of the patients were hypertensive. Sixty-six percent were on ACE treatment, 37% with β-blockers, and 23% with angiotensin II antagonists. The cause of renal failure was glomerulonephritis ($n = 9$), chronic pyelonephritis (6), systemic disease (4), polycystic kidney disease (2), nephroangiosclerosis (1), hereditary (1), nephronophthisis (1), and nephrocalcinosis (1). Ten patients had renal disease of unknown origin. The mean time on PD was 13.8 ± 6.6 months. Fifteen patients were on PD less than 1 year. Six patients were biopsied during their first 6 months (2, 3, 3, 4, 5, and 5 months, respectively) of treatment. None of these six patients had suffered peritonitis. The remaining nine patients were biopsied during their first 12 months on PD (7, 8, 9, 11, 11, 11, 11, 12, and 12 months). Two of these patients experienced an episode of peritonitis, which lasted 1 and 2 days, respectively. These 15 patients constituted the first year group. The other 20 patients were biopsied during their second year on PD, and the analysis was performed with the whole 35-patient group.

Ten parietal peritoneal biopsies obtained from nonrenal patients with no abdominal pathology were used as normal controls. Seven of them were renal donors and three autopsy cases.

Peritoneal samples

The parietal peritoneum samples were obtained during renal transplantation in most cases (29 patients, 77%) or during other abdominal interventions: nephrectomy ($n = 2$), catheter insertion (1), gastrectomy (1), omentectomy (1), and polypectomy (1).

Pathologic analysis

Biopsy collection and processing. Parietal peritoneal samples were obtained from the anterior abdominal wall. Each sample measured 10–25 × 10–25 mm. To avoid mesothelial artifactual detachment, they were carefully manipulated and immediately fixed with neutral-buffered 3.7% formalin (pH 7.3) for 12–24 h. To avoid retraction they were gently attached to a flat surface. After fixation, samples were cut and embedded in paraffin, and then cut into 3-μm sections. When preparing the paraffin blocks, special efforts were made to orientate the samples perpendicular to the cutting surface. Sections were stained with hematoxylin–eosin and Masson trichromic. For immunohistochemistry, paraffin sections were mounted on precoated slides, deparaffinized and rehydrated, and incubated with 3% hydrogen peroxide to block endogenous peroxidase activity. Antigen retrieval was performed using a citric acid solution (pH 6), which was heated with a microwave. Indirect immunohistochemical studies were performed by means of a dextran-polymer conjugate technique (EnVision+; Dakocytomation, Glostrup, Denmark). Monoclonal antibodies AE1/AE3 (cytokeratins) and α-smooth muscle actin (both from Dakocytomation) were used in this study. For visualization, diaminobenzidine was used as a chromogen.

Sample analysis. Morphological data regarding mesothelial status, thickness of submesothelial compact zone, hyalinizing vasculopathy, and vascular density were recorded. Density of MCs was measured using a semiquantitative scale (grade 3, normal; grade 0, total absence). The thickness of the compact zone was measured with a micrometer ocular. The mean of three different measures of representative zones was obtained: when less than 150 μm, it was considered normal; if between 150 and 350 μm, it was considered as a moderate thickening; results greater than 350 μm were regarded as intense thickening. Using immunohistochemistry, we explored the presence of submesothelial cytokeratin+ fibroblast-like cells and α-smooth muscle actin+ fibroblasts (myofibroblasts). EMT was

defined by the presence of submesothelial fibroblast-like cells expressing cytokeratin. The evaluation of vessels was performed using a method similar to that described by Numata *et al.*[30] Photomicrographs recording between 10 and 20 microscopy fields of each specimen ($\times 100$ magnification) were used for the quantitative analysis of vessels. The total number of cross-sections of vessels per peritoneal field (relative microvessel number) was examined. Hyalinizing vasculopathy was measured using the four grade system described by Honda *et al.*:[46] grade 0, no abnormalities; grade 1, mild thickening without stenosis of the lumen; grade 2, moderate thickening with partial luminal stenosis; and grade 3, intense thickening with marked stenosis and luminal distortion or complete occlusion.

Peritoneal function studies

A peritoneal transport kinetic study was performed to calculate the peritoneal Cr-MTAC ($\mathrm{ml\,min^{-1}}$), based on the creatinine dialysate/plasma ratios at five consecutive peritoneal 4-h dwells, using a previously described mathematical model.[3] The MTAC value is considered to represent exclusively the small solute diffusive transport across the membrane. To avoid a predefinition of normality, the obtained values were divided into quartiles. The highest quartile is considered that of the faster, higher transporters.

Net ultrafiltration rate (UF rate, ml) was estimated by the net negative balance (weighing bags during the kinetic study prior infusion and after drainage), using a 2-l hypertonic glucose exchange during the 4-h dwell time.

Ethical issues

The procedures were in accordance with the ethical standards of the Institutional Committee on Human Experimentation and with the Declaration of Helsinki Principles 1975 (and as revised in 1983). All patients were informed about the collection of cells from peritoneal effluent and peritoneal biopsy, and signed the informed consent.

Statistical analysis

Data are expressed in mean $\pm$ s.d. A *P*-value less than 0.05 is considered statistically significant. Comparisons of proportions between groups were made using the Fischer exact test and comparisons of means with the nonparametric Mann–Whitney *U*-test. Univariate and multivariate logistic regression analyses were used to investigate factors associated with the presence on peritoneal biopsy of the EMT, submesothelial fibrosis, and vasculopathy. We used the statistical program SPSS, version 11 (SSPS Inc., Chicago, IL, USA). The quartiles of peritoneal functional parameters have been related to histological data to compare the prevalence of each lesion in every quartile.

DISCLOSURE

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Capítulo 5. Transporte de pequeños solutos y agua a medio-largo plazo

6.1.5.1.- "Longevidad funcional del peritoneo humano: ¿Durante cuánto tiempo es posible la diálisis peritoneal? Resultados de un estudio prospectivo a medio y largo plazo"

Selgas R, Fernández Reyes MJ, Bosque E, Bajo MA, Borrego F, Jiménez C, del Peso G, de Álvaro F.

American Journal of Kidney Diseases 1994; 23(1): 64-73

Este trabajo responde a los objetivos 1, 2 y 3

Longevidad funcional del peritoneo humano: ¿Durante cuánto tiempo es posible la diálisis peritoneal? Resultados de un estudio prospectivo a medio y largo plazo.

Para que la DP pueda mantenerse a largo plazo, es necesario conservar un adecuado transporte peritoneal y es importante conocer qué cambios pueden producirse. El mejor método para evaluar el transporte difusivo es la medición de los coeficientes de transferencia de masas peritoneales (MTC).

Objetivo: Evaluar de forma prospectiva los cambios de la función peritoneal en pacientes con largas estancias en DP continua ambulatoria (DPCA).

Métodos: Se estudiaron 56 pacientes que comenzaron DPCA entre 1980 y 1988 y tenían al menos 3 años de seguimiento en la técnica. Todos disponían de al menos un estudio cinético anual (cálculo de UF con una fórmula estandarizada, y medición del MTC-urea y creatinina aplicando un modelo matemático bicompartimental). El MTC-urea no mostró cambios significativos (20.7±5.9 ml/min al año y 19.8±6 ml/min al quinto año), mientras que el MTC-creatinina aumentó de forma significativa en ese periodo. El descenso del cociente MTC-urea/MTC-creatinina se mostró un precoz y adecuado marcador de los cambios, incluso en presencia de valores individuales normales. La UF disminuyó significativamente (1800±530 ml/día a 1400±600 ml/día, $p<0.01$). Estos cambios se correlacionaron con un elevado número de días acumulados de inflamación peritoneal, por lo que se la presencia o no de peritonitis debe considerarse un marcador de supervivencia de la membrana peritoneal. Los cambios funcionales pueden estar relacionados con los efectos a nivel morfológico que provocan distintas agresiones en el peritoneo normal.

Conclusión: El transporte peritoneal de solutos y agua se mantiene estable tras más de 5 años de tratamiento con DP, salvo en los pacientes que acumulan una elevada tasa de peritonitis.

Functional Longevity of the Human Peritoneum: How Long Is Continuous Peritoneal Dialysis Possible? Results of a Prospective Medium Long-term Study

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● **Long-term peritoneal dialysis requires the maintenance of the transport function of the peritoneal membrane, and appropriate studies of possible changes are necessary. The quantification of peritoneal mass transfer coefficients (MTCs) has been judged to be the ideal method for the evaluation of peritoneal diffusion. The aim of the present study was to show the results of the prospective evaluations in long-term continuous ambulatory peritoneal dialysis patients. We have studied the clinical incidents and peritoneal function of 56 patients who started continuous ambulatory peritoneal dialysis between 1980 and 1988, and have completed at least 3 years of follow-up. Ultrafiltration capacity was calculated with a standardized formula. All patients were studied for peritoneal diffusion of urea and creatinine at least once a year. The evaluation consisted of a kinetic study done by means of a peritoneal equilibration curve for urea and creatinine, applying a bicompartmental mathematical model to calculate the MTCs. The sequential mean values for urea-MTC did not show significant changes over the observation period (20.7 ± 5.9 mL/min for the first year *v* 19.8 ± 6 mL/min for the fifth year). Creatinine-MTC values showed a significant increase over this period in the paired data analysis. The decrease of the urea-MTC to creatinine-MTC ratio may be an early and appropriate index for measuring these changes when the individual values are in the normal range. On the other hand, peritoneal ultrafiltration capacity significantly decreased over this period (1,800 ± 530 mL/d *v* 1,400 ± 600 mL/d, $P < 0.01$). The high rate of accumulated days of peritoneal inflammation was related to these significant changes, and thus may be proposed to be a good prognostic index of long-term peritoneal survival. These long-term functional changes might be related to the effect of injuries on the preservation of the normal peritoneal structure. We conclude that after 5 to 11 years, the human peritoneum shows functional stability (diffusion and water transport) in patients with low rates of peritoneal inflammation. With a few exceptions, represented by patients with a high rate of peritoneal inflammation, long-term peritoneal dialysis accomplished its newly entrusted task.**

INDEX WORDS: Peritoneal dialysis; continuous ambulatory peritoneal dialysis; peritoneal diffusion; peritoneal ultrafiltration capacity; peritoneal mass transfer coefficient; peritoneal inflammation.

LONG-TERM peritoneal dialysis requires the maintenance of the transport function of the peritoneal membrane, and appropriate studies of possible changes are necessary. The available data[1-3] suggest the functional stability of the peritoneal membrane, although this finding is not universal.[4] Patients who suffer conspicuous functional changes as well as those who are maintained on long-term continuous ambulatory peritoneal dialysis (CAPD) require greatest attention. Several methods have been introduced to evaluate peritoneal function: the peritoneal equilibration test,[5] the mass transfer coefficient (MTC) calculated by the Garred simplified formula,[6] and the classical peritoneal clearance test.[7]

A recent report has demonstrated the inaccuracy of MTCs quantified by Garred's formula,[8] particularly when there are high permeability situations. The quantification of peritoneal MTCs by complex mathematical modeling techniques has been judged to be the ideal method for the evaluation of peritoneal diffusion,[1-3,9-14] but the complexity of its quantification has prevented a more generalized use. For the last 10 years, we have evaluated the diffusive peritoneal capacity in all our CAPD patients using this methodology. The peritoneal ultrafiltration (UF) capacity, representing the water transport of each patient, also has been recorded.

The aim of this report is to show the results of the prospective evaluations in those patients maintained on CAPD for at least 3 years.

METHODS

Patients

We studied the clinical incidents and peritoneal function of 56 patients who started CAPD between 1980 and 1988, and completed at least 3 years of follow-up. Patients who

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stayed on CAPD for less than 3 years were excluded because these data already have been published.[1,3] The number of patients included in each annual period was 56 for the first 3 years and 44, 26, 13, 9, 7, 5, 3, and 1 for the following 8 years, respectively. The average length of follow-up was 5 years, and the total observation time was 276 patient-years. The average age of the patients starting CAPD was 48 ± 15 years (range, 16 to 73 years). Fifty-nine percent of the patients were females and 41% were males. Weight ranged from 42 to 98 kg (62 ± 10 kg) and height ranged from 140 to 179 cm (161 ± 8 cm). No pre-existing peritoneal damage was present in any patient. Peritoneal dialysate containing lactate was always used.

Renal failure was due to tubulointerstitial nephropathy in 18 patients, type I diabetes in seven, type II diabetes in six, chronic glomerulonephritis in eight, nephrosclerosis in six, polycystic kidney disease in four, unknown causes in four, and systemic, hereditary, and other causes in one each. Patients with type I diabetes were studied as an independent group since there is evidence that peritoneal peculiarities exist.[15] This latter group did not differ from the other patients in demographic characteristics, with the exception of a lower average age (33 *v* 50 years).

Peritonitis was recorded as both the number of episodes and the number of days of active peritoneal effluent inflammation (white blood cell count > 100/mL), which we have termed the "accumulated peritoneal inflammatory days" (APIDs). Patients were divided into two groups on the basis of a low (<11 days) and high (>10 days) APID to evaluate the effects of accumulated peritonitis. Changes in peritoneal function were assessed with respect to the number of peritoneal incidents in the former year.

A peritoneal resting period of 4 weeks was necessary in seven patients due to low UF and a significant increase in peritoneal diffusion. Recovery of UF capacity with a simultaneous decrease in creatinine MTC was observed at the end of this period.[16]

Studies of Peritoneal Function

Studies were performed under normal conditions in terms of blood chemistry, and the absence of protein and leukocytes in the peritoneal effluent. Otherwise, the procedure was delayed for a minimum of 4 weeks (ie, after episodes of peritonitis).

Convective function. Actual daily UF capacity was calculated by measuring the net negative balance of fluid under a rigid dialysis schedule: three 2-L exchanges with 1.5% dextrose and one 2-L exchange with 4.25% dextrose in the same day. Monthly values reflecting the average standardized daily UF were then obtained. For patients not using such a rigid exchange schedule, an appropriate calculation using the real figures under equivalent conditions on dwell times and dextrose content of dialysate was performed. Finally, the UF capacity for each annual period was calculated, averaging the 12 monthly averaged determinations.

Diffusive function. All patients were studied for peritoneal diffusion of urea and creatinine at least once a year.[11-14] Because we started calculating the mathematical model in 1981, only 43 patients were studied in basal conditions, that is, during their first 2 weeks on CAPD. Until the third year of follow-up, at least three observations were available for each patient. The number of tests performed for the following 8 years was 32, 18, 10, 7, 4, 4, 1, and 1, respectively.

The evaluation consisted of a kinetic study done by means of a peritoneal equilibration curve for urea and creatinine with dextrose 1.5% peritoneal fluid and taking effluent samples every 30 or 60 minutes. The data were completed with residual renal clearance and 24-hour solute generations. A bicompartmental mathematical model was applied (Appendix).[9-14] The MTC value was expressed as milliliters per minute.

Urea-MTC to creatinine-MTC ratio. We have defined the urea-MTC to creatinine-MTC ratio as an index of the proportionality between the transports of two molecules with different molecular weights.

Statistical Analysis

Each patient has been considered as his or her own control. A paired Student's *t*-test was used to compare the annual data. These results are shown in Tables 1, 2, and 3. The results not included in these tables (ie, after the seventh year) correspond to seven patients with the longest observation period and may be seen in Figs 1 through 5.

The sequential averaged data for the overall period and the whole series (from baseline to seventh year) were compared by mean of an ANOVA-1 test. These results are displayed in Figs 1 through 5.

To compare the different groups of patients, Student's test for unpaired data was performed. Finally, a regression analysis was done to establish the relationship between MTCs or UF and other parameters of the study. $P < 0.05$ was considered statistically significant. However, to show those mean values of marginal statistical significance, we also have indicated in the tables those probability values less than 0.1.

RESULTS

Five patients were transferred to hemodialysis due to membrane failure, which is defined as a degree of UF (four cases) or diffusion (one case) insufficient to maintain an adequate clinical condition. In all these cases the peritoneal functional change appeared after severe peritonitis or several bouts of peritonitis concentrated in short periods of time. The data of these patients have been included in the analysis.

The sequential mean values for urea-MTC are shown in Fig 1. The ANOVA test did not show significant changes over the observation period (20.7 ± 5.9 mL/min for the first year *v* 19.8 ± 6 mL/min for the fifth year). The paired data analysis confirmed these features because no significant differences appeared. Likewise, the ANOVA test for creatinine-MTC values did not show significant differences over the same period (9.3 ± 3.4 mL/min for the first year *v* 10.9 ± 3.1 mL/min for the fifth year) (Fig 2). However, the paired data study showed significant differences between values during the first 3 years and that obtained during the fifth, sixth, and seventh years,

Table 1. Results of the Paired Data Analysis for Creatinine-Mass Transfer Coefficient

	Year						
	1	2	3	4	5	6	7
Baseline	10.1 ± 6.2 9.5 ± 3.4 (41)	10.3 ± 6.4 9.4 ± 3.6 (39)	10.1 ± 6.1 10.7 ± 4.1 (38)	10.0 ± 6.0 10.8 ± 3.1 (23)	9.1 ± 5.2*** 12.9 ± 3.7 (12)	7.8 ± 3.0 11.1 ± 2.3 (5)	ISS
Year 1	XXX	9.4 ± 3.6 9.0 ± 3.7 (49)	9.4 ± 3.5** 10.6 ± 5.1 (48)	9.1 ± 3.5 10.4 ± 3.6 (30)	8.8 ± 2.8* 10.9 ± 4.4 (18)	8.2 ± 2.9* 10.4 ± 3.3 (10)	ISS
Year 2		XXX	8.8 ± 3.5** 10.5 ± 5.1 (47)	8.5 ± 4** 10.4 ± 3.5 (35)	8.1 ± 3** 10.7 ± 4.5 (17)	6.4 ± 2** 10.1 ± 3.3 (9)	6.6 ± 2.3** 10.8 ± 3.3 (6)
Year 3			XXX	10.2 ± 5.6 10.4 ± 3.5 (31)	8.6 ± 2.8** 10.8 ± 4.4 (18)	7.7 ± 3** 10.4 ± 3.2 (10)	8.1 ± 3.6 10.9 ± 3.1 (7)
Year 4				XXX	10.1 ± 3.3 10.9 ± 4.4 (18)	9.1 ± 3.5* 10.4 ± 3.3 (10)	9.0 ± 3 10.9 ± 3.1 (7)
Year 5					XXX	9.2 ± 3.7 10.4 ± 3.3 (10)	10.3 ± 3.5 10.9 ± 3.1 (7)
Year 6						XXX	10.5 ± 3.6 10.9 ± 3.1 (7)

NOTE. The numbers in parentheses indicate the number of observations for each paired analysis. Data are presented as mean ± SD. The data for paired analyses are displayed as follows: the upper data correspond to the year in the vertical column and the lower data correspond to the year in the horizontal row.

Abbreviation: ISS, insufficient sample size.

Statistical significance: ***$P < 0.01$, **$P < 0.05$, and *$P < 0.1$.

the latter always being higher (Table 1). The most remarkable and homogeneous differences existed between the second-year values and the rest of the study. After the fourth year the values remained stable. Figure 3 shows the urea and creatinine MTC values for each patient with more than 6 years of follow-up.

On the other hand, peritoneal ultrafiltration capacity (Fig 4) significantly decreased over the 5-year period (1,800 ± 530 mL/d *v* 1,400 ± 600 mL/d $P < 0.01$). The decrease is even higher when the baseline values are compared with those in the eighth year (1,050 ± 500 mL/d). Table 2 displays the statistically significant differences obtained by paired data analysis on UF capacity during the course of the study; no significant changes appeared until the fourth year, at which time a decrease was registered with respect to the initial values. Thereafter, UF continued to diminish and a significant decrease was also registered between the fifth and eighth years.

Figure 5 shows the mean values for the urea-MTC to creatinine-MTC ratio. There is a significant decrease during the period studied, the ratio

Table 2. Results of the Paired Data Analysis for Ultrafiltration Capacity (1/d)

	Year							
	1	2	3	4	5	6	7	8
Baseline	1.8 ± 0.5 1.9 ± 0.5 (56)	1.8 ± 0.5 1.8 ± 0.6 (56)	1.8 ± 0.5 1.8 ± 0.6 (56)	1.8 ± 0.5 1.7 ± 0.7 (44)	1.8 ± 0.6 1.6 ± 0.6 (26)	1.9 ± 0.5** 1.4 ± 0.5 (13)	2.0 ± 0.4*** 1.2 ± 0.4 (9)	2.0 ± 0.5*** 1.0 ± 0.6 (7)
Year 1	XXX	1.9 ± 0.5 1.9 ± 0.6 (56)	1.9 ± 0.5 1.8 ± 0.6 (56)	1.9 ± 0.4** 1.7 ± 0.7 (44)	1.9 ± 0.5** 1.6 ± 0.6 (26)	1.9 ± 0.3** 1.4 ± 0.5 (13)	2.0 ± 0.3** 1.2 ± 0.5 (9)	2.0 ± 0.3** 1.0 ± 0.6 (7)
Year 2		XXX	1.9 ± 0.6 1.8 ± 0.6 (56)	1.9 ± 0.5** 1.7 ± 0.7 (44)	2.0 ± 0.6** 1.6 ± 0.6 (26)	2.1 ± 0.4** 1.4 ± 0.5 (13)	2.1 ± 0.5** 1.2 ± 0.6 (9)	2.0 ± 0.3** 1.0 ± 0.6 (7)
Year 3			XXX	1.9 ± 0.6** 1.6 ± 0.7 (44)	2.0 ± 0.6** 1.6 ± 0.6 (26)	2.0 ± 0.4** 1.4 ± 0.5 (13)	2.1 ± 0.4** 1.2 ± 0.6 (9)	2.0 ± 0.4** 1.0 ± 0.6 (7)
Year 4				XXX	1.9 ± 0.6** 1.6 ± 0.6 (26)	1.9 ± 0.4** 1.4 ± 0.5 (13)	1.9 ± 0.5** 1.2 ± 0.6 (9)	1.9 ± 0.4** 1.1 ± 0.6 (7)
Year 5					XXX	1.7 ± 0.4** 1.4 ± 0.5 (13)	1.7 ± 0.4** 1.2 ± 0.6 (9)	1.7 ± 0.5** 1.0 ± 0.6 (7)

See Table 1 note for explanation of data.

Statistical significance: ***$P < 0.01$, **$P < 0.05$.

Table 3. Results of the Paired Data Analysis for the Urea-Mass Transfer Coefficient to Creatinine-Mass Transfer Coefficient Ratio

	Year						
	1	2	3	4	5	6	7
Baseline	2.7 ± 1.1**	2.7 ± 1.1**	2.6 ± 1.1**	2.6 ± 1.1**	2.9 ± 1.3**	2.7 ± 0.9	ISS
	2.3 ± 0.7 (41)	2.3 ± 0.7 (39)	2.0 ± 0.7 (38)	1.9 ± 0.5 (23)	1.8 ± 0.3 (12)	1.8 ± 0.4 (5)	
Year 1	XXX	2.4 ± 0.9	2.4 ± 0.9*	2.4 ± 0.8*	2.5 ± 1**	2.4 ± 1.3	2.6 ± 1.6
		2.4 ± 0.9 (49)	2.2 ± 0.8 (48)	2 ± 0.6 (18)	1.9 ± 0.3 (18)	1.9 ± 0.4 (10)	1.9 ± 0.6 (7)
Year 2		XXX	2.5 ± 0.9**	2.5 ± 0.9**	2.5 ± 1**	2.9 ± 1.2**	3 ± 1.1
			2.1 ± 0.7 (47)	2.0 ± 0.6 (31)	1.9 ± 0.3 (17)	1.9 ± 0.4 (9)	2 ± 0.7 (6)
Year 3			XXX	2.2 ± 0.7**	2.3 ± 0.6**	2.6 ± 0.8**	2.4 ± 0.6**
				2.0 ± 0.6 (31)	1.9 ± 0.3 (18)	1.9 ± 0.4 (10)	1.9 ± 0.6 (7)
Year 4				XXX	2 ± 0.5	2.2 ± 0.6	2.1 ± 0.4
					1.9 ± 0.3 (18)	1.9 ± 0.4 (10)	1.9 ± 0.6 (7)
Year 5					XXX	2 ± 0.4	2.1 ± 0.3
						1.9 ± 0.4 (10)	1.9 ± 0.6 (7)
Year 6						XXX	2 ± 0.4
							1.9 ± 0.6 (7)

See Table 1 note for explanation of data.
Statistical significance: ***$P < 0.01$, **$P < 0.05$, *$P < 0.1$.
Abbreviation: ISS, insufficient sample size.

being remarkably lower for the fifth and following years when compared with the baseline values (2.5 ± 0.8 *v* 1.75 ± 0.4, $P < 0.01$). Paired data analysis confirmed these results (Table 3). Significant differences between paired values of the MTCs ratio appeared during the first year on CAPD. During the first 5 years there was a continuous decrease in the MTC ratio. Thereafter, the value stabilized.

Regression Analysis

The linear correlation analyses for MTCs, MTC ratio, and UF are shown in Table 4. There was an inverse correlation between the creatinine-MTC and UF values, which disappeared when only values of creatinine-MTC higher than 12 mL/min were considered (from $r = -0.48$, $P < 0.05$ to $r = -0.06$, P = NS). A direct relationship between UF and the MTC ratio also was

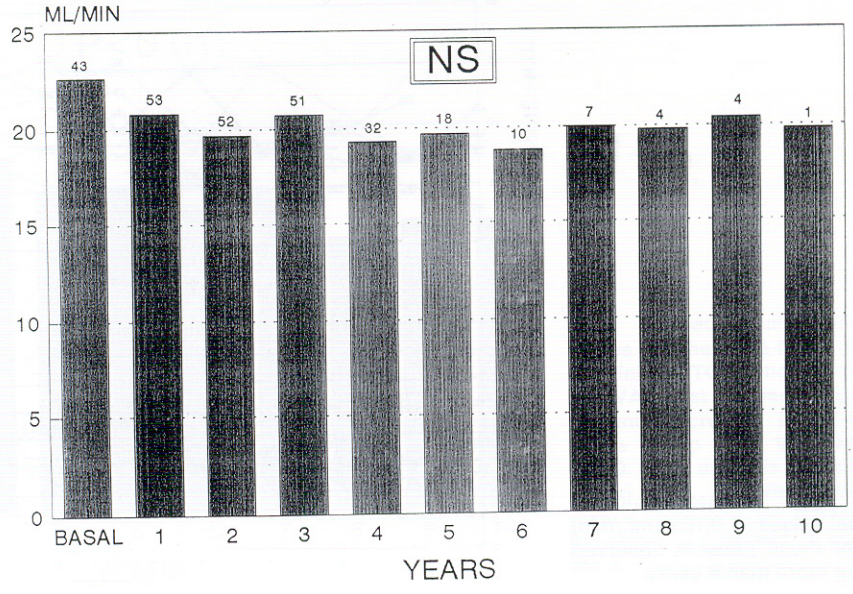

Fig 1. Sequential mean values of urea-MTCs. The ANOVA-1 test did not show statistically significant differences for the whole group over the studied period.

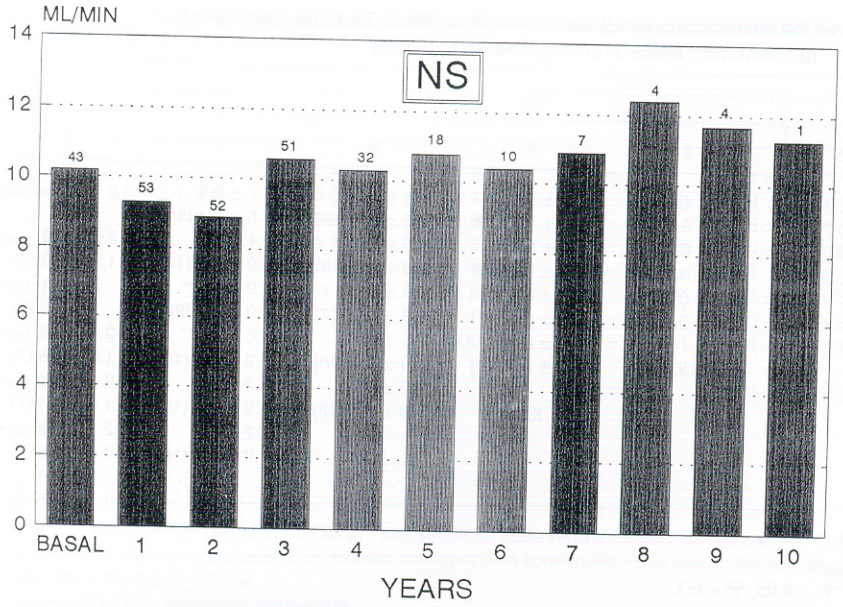

Fig 2. Sequential mean values of creatinine-MTC. The ANOVA-1 test did not show statistically significant differences for the whole group over the studied period. The analysis of the paired data, between each annual study and the following years, is displayed in Table 1.

found. The correlation between the MTC ratio and urea-MTC did not reach statistical significance, but there was a significant relationship between creatinine-MTC and the MTC ratio, both linear ($r = 0.59$) and polynomial ($r = -0.76$).

We found a poor correlation between baseline values and those subsequently obtained for the same patient. The values obtained after 1 year on CAPD appeared more stable and thus more representative.

There was no correlation between MTC values and the patient's body surface area or height for either the global data or the baseline values. The MTC ratio, however, was significantly correlated with the patient's height in the baseline study ($r = 0.55$, n = 43, $P < 0.05$). Correction of UF capacity according to body surface did not alter these correlations.

Peritonitis effect. Table 5 shows the linear correlation coefficients between the MTCs, UF, and MTC ratio and the years on CAPD in the two groups of patients defined by APID. The group with a lower incidence did not show a significant correlation between the time on CAPD

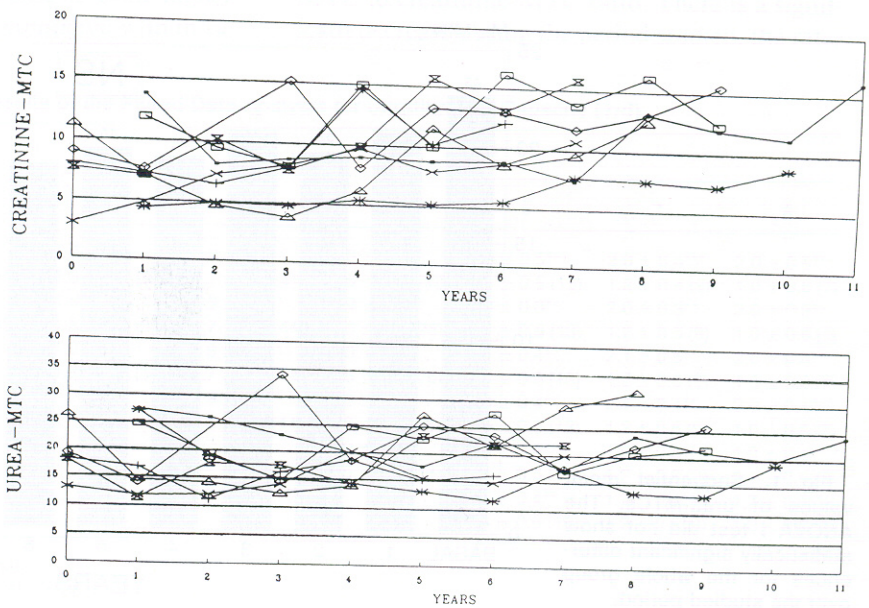

Fig 3. Sequential individual values of MTCs (mL/min) for those patients observed for more than 6 years.

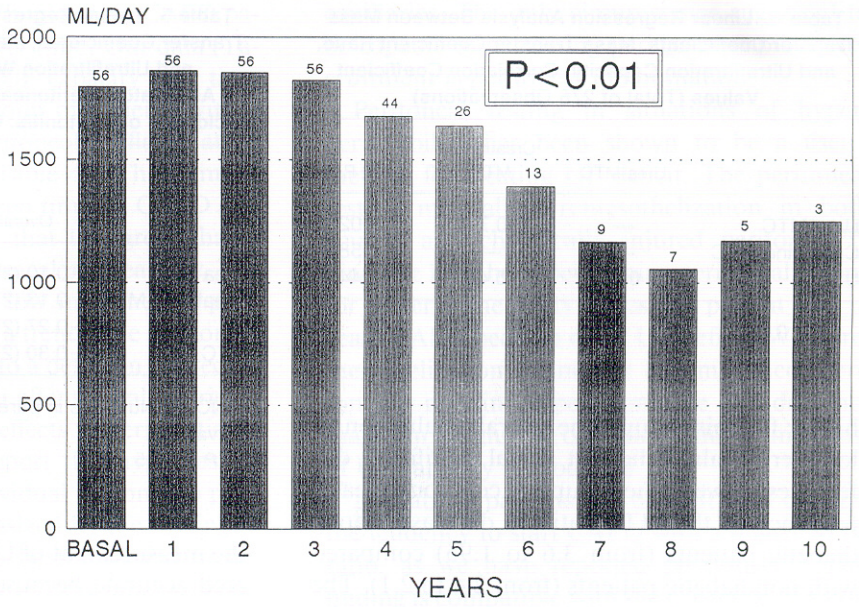

Fig 4. Sequential mean values of UF capacity. The ANOVA-1 test showed a significant decrease for the whole group over time on CAPD. The analysis of the paired data, between each annual study and the following years, is displayed in Table 2.

and UF capacity. The group with a higher incidence, however, showed a significant inverse correlation between these two parameters.

Creatinine-MTC was not related to time on CAPD in either group, but there was a tendency for the creatinine-MTC to increase in the higher incidence group, while no variation in the lower incidence group was observed. The value of the linear correlation coefficient between the MTC ratio and UF was lower in the higher incidence group than in the lower incidence group ($r = 0.33$ and $r = 0.47$, respectively). The correlation between creatinine-MTC and UF was similar for both groups ($r = 0.49$ and $r = 0.48$, respectively).

Peculiarities of Diabetic Patients

Mean basal values for type I diabetic and non-diabetic patients (type II included) are displayed in Table 6. The differences between the two groups did not reach statistical significance, probably due to the small number of diabetic patients, but the values of the MTC ratio were

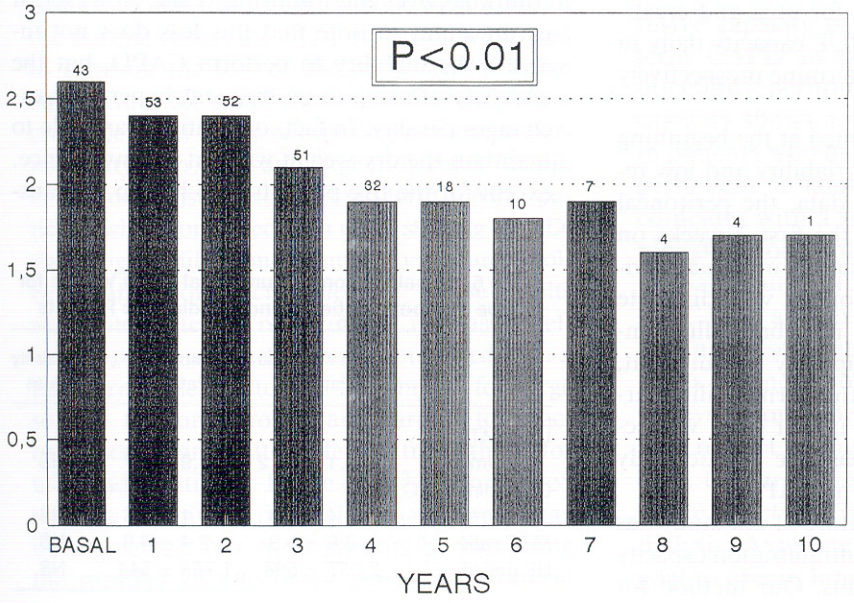

Fig 5. Sequential mean values of the urea-MTC to creatinine-MTC ratio. The ANOVA-1 test showed a significant decrease for the whole group over time on CAPD. The analysis of the paired data, between each annual study and the following years, is displayed in Table 3.

Table 4. Linear Regression Analysis Between Mass Transfer Coefficients, Mass Transfer Coefficient Ratio, and Ultrafiltration Capacity: Correlation Coefficient Values (Total of 276 Observations)

	Urea-MTC	Creatinine-MTC	MTC Ratio
Urea-MTC	—	0.71*	0.028
Creatinine-MTC	—	—	−0.58*
UF	−0.20*	−0.48*	0.44*

*$P < 0.05$.

Table 5. Linear Regression Analysis Between Mass Transfer Coefficients, Mass Transfer Coefficient Ratio, and Ultrafiltration With Years on Continuous Ambulatory Peritoneal Dialysis, According to the Incidence of Peritonitis: Correlation Coefficient Values

		Incidence	
	Overall	Low	High
Urea-MTC	−0.10 (276)	−0.19 (193)*	0.01 (82)
Creatinine-MTC	0.13 (276)*	−0.02 (193)	0.20 (82)
UF	−0.27 (276)*	−0.11 (193)	−0.36 (82)*
MTC ratio	−0.30 (276)*	−0.24 (193)*	−0.36 (82)*

NOTE. Numbers in parentheses indicate number of observations.
*$P < 0.05$.

higher for this group. The overall evaluation of long-term values did not reveal significant differences between the groups, except for a greater tendency of the MTC ratio to decrease among diabetic patients (from 3.6 to 1.95) compared with nondiabetic patients (from 2.4 to 2.1). The effect of peritonitis on diabetic patients was quite difficult to assess because only two were included in the higher incidence group. For these two patients the UF decrease had an earlier onset and appeared more rapidly than in the nondiabetic patients.

DISCUSSION

Long-term maintenance of CAPD entails a challenge to the peritoneal membrane. Dialysis may affect different peritoneal cells, particularly mesothelial cells, modifying their characteristics.[17] During the last 10 years, we have determined annually the MTCs for urea and creatinine. We also recorded the UF capacity daily in all our CAPD patients to determine prospectively the peritoneal function.

The MTC studies performed at the beginning of CAPD showed a large variability and low reliability. According to our data, the peritoneal kinetic study done during the first 2 weeks on CAPD gave poor representation of actual peritoneal function. The first contact with dialysate probably confers a special membrane-fluid interaction with variable capillary recruitment, which induces changes in the permeability/surface area disposal.[18] Based in our data, we recommend performing the baseline kinetic study at least 1 month after starting CAPD.

The most remarkable feature of our study has been the progressive loss of ultrafiltration capacity on long-term CAPD patients. Our method for the measurement of UF capacity may be considered accurate because it reflects the average of many measurements per year. The peritoneal ultrafiltration rate is the result of several forces (UF coefficient, glucose osmotic reflecting coefficient, glucose MTC, and peritoneal lymph flow).[19-22] It has been demonstrated that creatinine and glucose MTCs maintain a significant direct relationship,[23] reflecting that both transports, although in inverse sense, are closely related. As a consequence, UF capacity also is related to the peritoneal diffusion capacity of creatinine.[23] We have not found any significant change in UF during the first 4 years on CAPD; however, a significant loss unfortunately appears after this date and continues over the following years. However, it is important to note that this loss does not indicate the inability to perform CAPD, but the necessity of using more frequent hypertonic exchanges per day. In fact, our patients are able to maintain the dry weight without inconvenience, except for the five patients who had to be trans-

Table 6. Basal Peritoneal Functional Mean Values for Type I Diabetic Patients and Nondiabetic Patients

	Type I Diabetic Patients	Nondiabetic Patients	Probability Values
Urea-MTC (mL/min)	26.1 ± 11.2	21.9 ± 6.7	NS
Creatinine-MTC (mL/min)	10.4 ± 8.9	10.1 ± 5.6	NS
MTC ratio	3.6 ± 1.9	2.4 ± 0.8	NS
UF (mL/d)	2,057 ± 594	1,764 ± 544	NS

ferred to hemodialysis. Why do some CAPD patients lose UF capacity over time? Our data (Table 5) show a decline of UF capacity over time on CAPD only in the group of patients with a high incidence of peritoneal inflammation (APID) (right column, Table 5). The demonstrated relationship between time on CAPD and creatinine-MTC suggests that this group has a tendency to increase peritoneal permeability. The group of patients with a low incidence of peritonitis did not show such a trend. The peritoneal UF loss might be related to a defect in the recovery of the mesothelial layer after several injuries.[17] Data showing the direct effects of peritonitis on the mesothelial cells support this hypothesis.[24] The relevance of the individual response to peritoneal infection is emphasized by the finding in five patients who had to be transferred to hemodialysis. No consistent circumstance or etiology could be found to explain their special behavior.[4]

The increment of creatinine-MTC over time on CAPD, without a simultaneous increment of urea-MTC, may reflect structural changes in the peritoneal components involved. Since the larger the molecule, the greater the dependence on intrinsic membrane permeability, the smaller the molecule, the less the dependence on permeability. For small molecules, transport across the peritoneum depends fundamentally on the available area; thus, increments in permeability affect small molecules less than large molecules. In this context, when the values of both MTCs are in the normal range, the MTC ratio might be used to measure the loss of the peritoneal diffusive equilibrium for small molecules. Recently, in a cross-sectional study comparing peritoneal permeability with small and large molecules, Struijk et al[18] demonstrated that long-term CAPD patients, when compared with those starting CAPD, show higher diffusion for small to medium molecules than for macromolecules. These results were interpreted as reflecting an increase in effective peritoneal surface area, combined with a less permeable peritoneal membrane for large solutes. Peritoneal routes and barriers for macromolecules are quite different from those for urea and creatinine. Rippe et al[25] demonstrated that the effective peritoneal exchange area may be augmented in CAPD patients by increasing the number of effectively perfused peritoneal capillaries. The only circumstance suggested to be capable of increasing peritoneal capillary recruitment is peritoneal inflammation.

Peritoneal resting in situations of hyperpermeability has been shown to be a useful method for treating UF deficit. The peritoneal resting may allow remesothelization in both acutely and chronically injured peritonei.[16,17] Since we have been performing peritoneal resting for hyperpermeability states, no patient had to cease CAPD because of an UF deficit. When remesothelization cannot be accomplished, peritoneum remains hyperpermeable and does not maintain the glucose osmotic gradient, and a loss of UF capacity is produced.

In diabetic patients our current data confirm the tendency to start CAPD with a higher MTC ratio, as we have reported previously.[15] This finding is compatible with the existence of a normal peritoneal surface area with lower relative permeability. Glycation of peritoneal capillary basement membrane is a possible explanation. This peculiar baseline functionalism tends to disappear during CAPD treatment. We do not have enough data to definitively analyze the long-term peritoneal response in diabetic patients who have suffered a high peritonitis rate. However, individual data suggest that diabetic peritoneum may be more susceptible to inflammatory injuries and changes in UF appear earlier.

The main conclusion from the present study is the absence of significant changes in the diffusive capacity of the peritoneum after long-term CAPD in patients with a low rate of infectious peritoneal injuries. Ultrafiltration capacity shows a decline after the fourth year in patients who have a large number of days of peritoneal inflammation. This phenomenon coincides with a significant increase in creatinine diffusion, without a simultaneous increase in that of urea. The urea-MTC to creatinine-MTC ratio appears to be a useful tool in interpreting these peritoneal changes. Diabetic patients did not show significant differences on the evolution of peritoneal transport capacities with respect to nondiabetic patients. In conclusion, the human peritoneum shows an appropriate functionalism for long-term peritoneal dialysis. Avoiding peritonitis seems to be the goal to obtain long-term stability.

APPENDIX

Solute kinetics can be described by a single pool constant volume model for the patient and by a variable volume dialysate pool as follows:

Mass transfer =

$$d(VDCD)/dt = MTC(CB - CD) + TrQuCB \quad (1)$$

where V = volume of dialysate or distribution volume for blood; C = concentration; D = dialysate. B = blood; t = time; MTC = mass transfer coefficient; Tr = exp $(-0.0609 \times MW^{1/3})$ = transmittance coefficient (1 for urea and creatinine); and Qu = ultrafiltration rate (dVD/dt).

A total mass balance yields

$$VDCD + VBCB = Gt - KrCBt + CB_0VB + CD_0VD_0 \quad (2)$$

where G = generation rate; Kr = residual renal function; O = initial values; and VB = $^4/_7$ body weight.

Solving equation 2 for VB assuming constant and small variation in CB with substitution in equation 1 yields

$$dCD/dt = 1/VD[\alpha1\alpha2 + \alpha1Gt - (MTC + Qu + \alpha1VD)CD] \quad (3)$$

$$\alpha1 = MTC + QuTr/VB + Krt \quad (4)$$

$$\alpha2 = CB_0VB + CD_0VD_0 \quad (5)$$

Since VD and Qu are functions of time, a method of solution is approximation of the differential by finite difference numerical techniques. This results in

$$CD_{(n+1)} = It/VDn[\alpha1\alpha2 + \alpha1Gtn - (MTC + Qu + \alpha1VDn)CDn] + CDn \quad (6)$$

where $CD_1 = CD_0$ (initial condition) and It = increment of time.

Equation 6 is fit to a concentration profile in the least-squares sense where the error is given by S:

$$S = \sum_{i=1}^{j} \epsilon(CD_i^1 - CD_i)^2 \text{ (ith calculated value} - \text{ith data value).}$$

The best fit to the data is obtained for

$$dS/dX = 0$$

where X is MTC in this case.

Integration of the differential equations and determination of MTC was carried out by the minimization of the fourth-order quadratic error method of Runge and Kutta, with adaptation of the integration interval (subroutine DEF of the IBM PL-MAT Library). The minimization was carried out through the Powell method (subroutine FMND of the same library). The integration interval was 1 minute. The number of iterations for achieving the minimum was four.

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6.1.5.2.- “La influencia de las características del transporte peritoneal inicial, la inflamación y la elevada exposición a la glucosa en el pronóstico de la función de la membrana peritoneal”

Fernández-Reyes MJ, Bajo MA, del Peso G, Ossorio M, Díaz R, Carretero B, Selgas R.

Peritoneal Dialysis International 2012; 32(6): 636-644

Este trabajo responde a los objetivos 1, 2 y 3

La influencia de las características del transporte peritoneal inicial, la inflamación y la elevada exposición a la glucosa en el pronóstico de la función de la membrana peritoneal.

El alto transporte adquirido con el tiempo en DP es un proceso patológico secundario a la exposición del peritoneo a soluciones bioincompatibles. Tiene repercusión clínica y por ello debe ser evitado.

Objetivo: Analizar la influencia que tienen las características del transporte peritoneal al inicio de la DP en el pronóstico funcional peritoneal, y evaluar la repercusión de las peritonitis y de la elevada exposición a la glucosa en función del tipo de transporte inicial o del momento en el que sucedan.

Métodos: Se incluyen 275 pacientes en DP que dispongan de al menos dos estudios de función peritoneal (basal y al año), realizados con un intercambio de 4 horas de permanencia con glucosa (1.5% en 1981–1990, 2.27% en 1991–2002). Se calcularon los coeficientes de transferencia de masa de urea y creatinina (ml/min) según un modelo matemático complejo previamente ya descrito.

Resultados: El pronóstico de la membrana y la supervivencia en la técnica fueron independientes del tipo de transporte peritoneal inicial. Tanto el alto transporte peritoneal como el fallo de UF son reversibles, en ausencia de peritonitis o alta exposición a glucosa durante los primeros años en DP. El primer año en DP es determinante para el futuro de la membrana peritoneal, y el mejor predictor de la función peritoneal futura es el MTC de creatinina al año. A partir de los cinco años en DP, el transporte peritoneal suele aumentar y la UF disminuir. La icodextrina minimiza estas alteraciones.

Conclusiones: El pronóstico del funcionamiento de la membrana peritoneal es independiente de las características funcionales basales. El alto transporte y el déficit de UF intrínsecos son reversible si se evitan en los primeros años en DP las peritonitis y alta exposición a glucosa. El uso de icodextrina puede contribuir a evitar el abuso de glucosa y por tanto proteger la membrana peritoneal.

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THE INFLUENCE OF INITIAL PERITONEAL TRANSPORT CHARACTERISTICS, INFLAMMATION, AND HIGH GLUCOSE EXPOSURE ON PROGNOSIS FOR PERITONEAL MEMBRANE FUNCTION

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♦ ***Background:*** **Fast transport status, acquired with time on peritoneal dialysis (PD), is a pathology induced by peritoneal exposure to bioincompatible solutions. Fast transport has important clinical consequences and should be prevented.**

♦ ***Objective:*** **We analyzed the repercussions of initial peritoneal transport characteristics on the prognosis for peritoneal membrane function, and also whether the influence of peritonitis and high exposure to glucose are different according to the initial peritoneal transport characteristics or the moment when such events occur.**

♦ ***Methods:*** **The study included 275 peritoneal dialysis patients with at least 2 peritoneal function studies (at baseline and 1 year). Peritoneal kinetic studies were performed at baseline and annually. Those studies consist of a 4-hour dwell with glucose (1.5% during 1981 – 1990, and 2.27% during 1991 – 2002) to calculate the peritoneal mass transfer coefficients of urea and creatinine (milliliters per minute) using a previously described mathematical model.**

♦ ***Results:*** **Membrane prognosis and technique survival were independent of baseline transport characteristics. Fast transport and ultrafiltration (UF) failure are reversible conditions, provided that peritonitis and high glucose exposure are avoided during the early dialysis period. The first year on PD is a main determining factor for the membrane's future, and the mass transfer coefficient of creatinine at year 1 is the best functional predictor of future PD history. After 5 years on dialysis, permeability frequently increases, and UF decreases. Icodextrin is associated with peritoneal protection.**

♦ ***Conclusions:*** **Peritoneal membrane prognosis is independent of baseline transport characteristics. Intrinsic fast transport and low UF are reversible conditions when peritonitis and high glucose exposure are avoided during the early dialysis period. Icodextrin helps in glucose avoidance and is associated with peritoneal protection.**

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KEY WORDS: Peritoneal transport outcome; bioincompatible solution; peritonitis.

As a kidney substitutive treatment, peritoneal dialysis (PD) has been shown to achieve results similar to those achieved with hemodialysis (HD) in the medium term (1–4). However, the ability to use a living tissue for dialysis is determined by intrinsic membrane differences, including structural characteristics and reactions to inflammation and to exposure to bioincompatible fluids. In fact, during the era of bioincompatible solutions, 20% – 30% of patients experienced an increase in peritoneal permeability to small solutes, accompanied by a decrease in ultrafiltration (UF) capacity, after 3 – 4 years on PD (5–9). This phenomenon, called "acquired fast transport" (FT), has been attributed to various factors, mainly peritoneal inflammation (5) and solutions with an acidic pH or a high glucose concentration (8–10). It is also associated with dialysis-induced anatomic changes such as epithelial-to-mesenchymal transition of mesothelial cells (11), submesothelial fibrosis, and angiogenesis (12). However, most of the basal or acquired variability in peritoneal transport remains unexplained, and genetic or intrinsic factors may play an important role (13).

Controversy surrounds the influence of intrinsic (demonstrable at PD initiation) conditions on patient and membrane survival. Earlier studies conducted by our group showed a large variability in peritoneal transport—both for water and small solutes—at PD start (14), with no effects on peritoneal or patient survival (15). Those findings contrast with data presented by other authors that show a relationship between faster initial transport and poorer survival (16). Also, our data and those of others have shown that initial FT values decline toward normal values over the first dialysis year (6,17,18); however, other authors have found a very early and persistent increase in peritoneal permeability (9). A recent review divided inherent membrane failure in two

types, one associated with vasculopathy and endothelial dysfunction and related to comorbidity; and the other associated with a large peritoneal surface area. The prognosis is usually poor in the former case and good in the latter (19).

Our objectives in the present study were to analyze the repercussions of initial peritoneal transport characteristics on the prognosis for peritoneal membrane function, and also whether the influence of peritonitis and high exposure to glucose are different according to the initial peritoneal transport characteristics or the moment when such events occur. A secondary aim was to discover whether clinical maneuvers such as use of automated PD (APD) and icodextrin prevent the development of acquired FT.

METHODS

PATIENTS

Initially, the study encompassed 403 incident patients from the La Paz University Hospital PD program who were treated between 1980 and 2002. All had a peritoneal function study performed during the first 2 months on PD. Exclusion criteria were age under 18 or over 85 years; previous time on PD or kidney transplantation; and use of PD fluids low in glucose degradation products or containing bicarbonate or amino acids. Patients using an icodextrin bag daily either from the beginning or at any time were included. Table 1 shows the demographic, clinical, and baseline peritoneal characteristics of those 403 patients.

Peritoneal kinetic studies were performed at baseline and annually. The patients included in each annual period from baseline to dialysis year 9 numbered 403, 275, 170, 101, 67, 38, 27, 16, 12, and 9. Because the purpose of the present study was to investigate peritoneal function outcome, we analyzed the 275 patients who had at least 2 peritoneal function studies (baseline and year 1). All results and analysis refer to that group, which was not demographically and clinically different from the entire group of patients (Table 1).

Peritonitis episodes, registered both as episodes and as number of days of peritoneal inflammation, were recorded for each dialysis year to evaluate any direct effects on the subsequent peritoneal kinetic study. To evaluate the effect of PD on peritoneal function in the absence of peritonitis, patients were studied until a first peritonitis episode.

To quantify the effect of peritoneal glucose load on peritoneal function, we calculated the percentage of bags with 2.27% and 3.86% glucose content. Use of 2.27% bags exceeding 75% or use of 3.86% bags exceeding 25% was considered to be high glucose exposure (HGE); this level of exposure was expressly selected in all our cases to exceed the more than 6% described by Davies as high exposure (9). To evaluate the direct effect of such exposure on a subsequent peritoneal kinetic study, data were recorded for each dialysis year.

From PD start, only 21 and 14 patients used icodextrin or APD respectively, but changes in PD modality [APD to or from continuous ambulatory PD (CAPD)] and use of icodextrin were recorded in subsequent dialysis years. At years 1, 2, 3, 4, and 5 respectively, patients using icodextrin numbered 41, 39, 18, 14, and 6, and those using APD numbered 47, 44, 20, 19, and 11.

TABLE 1
Demographic, Clinical, and Baseline Peritoneal Function Characteristics[a] of the Study Patients

Variable	Overall	Patient groups Included in follow-up study	With 5 years on PD
Patients (*n*)	403	275	38
Mean age (years)	52.9±15.6	52.5±15.4	48.9±14.6
Sex (% men)	52.9	50.5	44.7
With diabetes (%)	25.8	23.2	26.4
Coming from HD [*n* (%)]	52 (12.9)	34 (12.4)	4(10.5)
MTC creatinine (mL/min)	10.6±5.0	10.7±5.3	10.8±5.9
MTC urea (mL/min)	23.3±7.5	23.2±7.9	23.4±7.4
Ultrafiltration capacity (mL/4 h)	881±351	899±397	930±400
MTC ratio (urea/creatinine)	2.5±1.0	2.4±0.9	2.5±1.1
Residual renal function (mL/min)	4.1±2.8	4±2.8	3.4±2.6

PD = peritoneal dialysis; HD = hemodialysis; MTC = mass transfer coefficient.
[a] No variable was significantly different between the groups.

PERITONEAL FUNCTION STUDIES

Each peritoneal transport kinetic study consisted of a 4-hour glucose dwell (1.5%, 1981–1990; 2.27%, 1991–2002), with 6 peritoneal effluent samples (at 0, 30, 60, 120, 180, and 240 minutes) and 1 blood sample being taken for calculation of the peritoneal mass transfer coefficient (MTC) of urea and creatinine (Cr) in milliliters per minute using a previously described mathematical model (5). All studies were performed while the patients were stable on PD, not having experienced a peritonitis episode during at least the preceding 4 weeks.

Daily UF capacity was estimated by weighted negative balance between infused and drained bags under a standardized dialysis schedule: three 2-L exchanges with 1.5% glucose, and one 2-L exchange with 4.25% glucose on the same day. For patients not using that schedule, we developed a nomogram for use by all members of the PD unit to calculate UF capacity under equivalent exchange conditions for dwell and glucose level; that methodology has already been described by our group (5).

STATISTICAL ANALYSIS

Using quartile distribution, the MTC Cr was divided into low [L (slow)] transporters (<7.2 mL/min; mean: 5.5 ± 1.3 mL/min; $n = 72$); low-average (LA; 7.2 – 9.7 mL/min; mean: 8.4 ± 0.7 mL/min; $n = 66$); high-average (HA; 9.7 – 12.9 mL/min; mean: 11.3 ± 0.9 mL/min; $n = 70$), and high [H (fast)] transporters (>12.9 mL/min; mean: 17.8 ± 5.3 mL/min; $n = 67$).

Values are expressed as percentages and means ± standard deviations, or as medians and ranges when distribution is not normal. Percentages were compared using the chi-square test; means, the Student t-test for unpaired data; and medians, the Mann–Whitney test. To analyze the simultaneous effect of several variables on UF failure, a Cox proportional hazards analysis was applied. A value of $p < 0.05$ was considered statistically significant.

Because of the progressive introduction of APD (starting in 1995) and icodextrin (starting in 1998) at our facility, and a change in the glucose level of the PD fluid used for peritoneal kinetic studies (to 2.27% from 1.36%), an analysis by dialysis vintage was performed.

Comparisons between groups were conducted by fitting a linear regression to the repeated measures in each group and by comparing the slopes of the corresponding regression lines (post-hoc Bonferroni test). To study the variable outcomes, a mixed-model analysis that allows for the evaluation of co-variables (the appearance of peritonitis, changes in intraperitoneal glucose load, and use of icodextrin during the interval) was applied. The linear mixed-model analysis, with its unstructured covariance matrix for quantitative variables (UF and creatinine transport parameters) in the framework of generalized mixed-models, allowed for the study of the complete outcome of each variable over time. The results should be interpreted as follows:

- "Significant group" means that having or not having peritonitis is different, but that the variation over time is not significantly different (parallelism is maintained).
- "Significant time" means that time affects both groups similarly.
- "Significant model" means that the group–time interaction is $p < 0.01$.

For the statistical analysis, we used the SPSS software program (version 15: SPSS, Chicago, IL, USA).

RESULTS

The median follow-up time for the complete series was 30 months (range: 12 – 193 months). During follow-up, 16 patients were transferred to hemodialysis because of UF failure. Comparing those patients with the rest of the cohort, we found a significant difference in median follow-up [48 months (range: 16 – 127 months) vs 30 months (range: 12 – 193 months), $p < 0.05$] and median accumulated days of active peritonitis [9.5 months (range: 0 – 32 months) vs 2 months (range: 0 – 36 months), $p < 0.05$]. At baseline, no differences in MTC Cr, UF capacity, and residual renal function (RRF) were found. A Cox proportional hazards analysis that included the results of peritoneal transport kinetic studies at baseline and year 1, RRF, and peritonitis episodes and glucose overexposure during year 1, showed that only the 1-year MTC Cr was significantly associated with UF failure (Table 2). The table omits data about year 1 MTC urea, UF, and RRF because those variables do not fit into the model when year 1 MTC Cr is included (relative risk: 1.112; 95% confidence interval: 1.013 to 1.219; $p = 0.025$).

MTC CR OUTCOME

Figure 1 shows the MTC Cr outcome over the study period. Similar results are seen for patients reaching 1, 2, 3, 4, and 5 years on dialysis [Figure 1(A)]. In all groups, we observed a decrease in MTC Cr from baseline to year 2 and a general increase after 4 – 5 years on PD. Figure 1(B) shows the MTC Cr outcome for the 38 patients who reached 5 years on PD. In their case, the year 1 to year 2 decrease was nonsignificant relative to baseline, but a statistically

TABLE 2
Cox Proportional Hazards Analysis for Ultrafiltration Failure

Variable	Relative risk	95% Confidence limits Inferior	Superior	p Value
Age (per year)	0.999	0.965	1.034	NS
MTC creatinine (mL/min) at baseline	1.056	0.985	1.132	NS
MTC urea (mL/min) at baseline	1.052	0.999	1.107	NS
Residual renal function (mL/min) at baseline	0.972	0.796	1.187	NS
Ultrafiltration (3.86 L) at baseline	1	0.999	1.001	NS
MTC creatinine (mL/min), first year	1.112	1.013	1.219	0.025
No high-glucose exposure, first year	0.439	0.161	1.194	NS
Icodextrin at baseline	0.539	0.062	4.687	NS
Peritonitis (days), first year	1.013	0.919	1.117	NS

NS = nonsignificant; MTC = mass transfer coefficient.

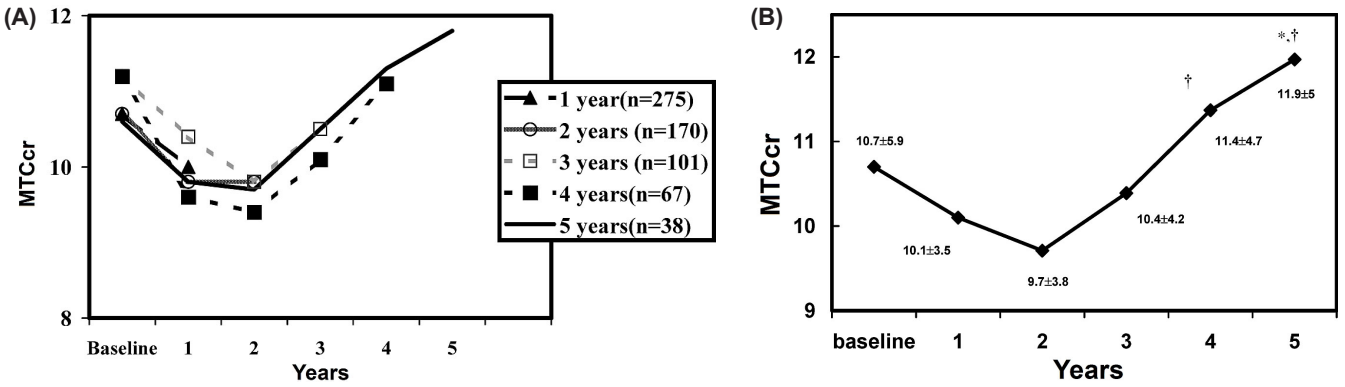


Figure 1 — Mass transfer coefficient of creatinine (MTCcr) (A) in all patients, and (B) in the 38 patients who reached 5 years on peritoneal dialysis. In the latter patients, the decline in MTCcr from year 1 to year 2 was nonsignificant relative to baseline. * Statistically significant increase compared with year 1. † Statistically significant increase compared with year 2.

significant increase was observed from year 4 to year 5 (from year 1 to year 5: $p = 0.036$; from year 2 to years 4 and 5: $p = 0.037$ and $p = 0.007$ respectively).

UF CAPACITY OUTCOME

Figure 2 shows the UF capacity outcome. In mirror image relative to the MTC Cr, a nonsignificant increase in UF capacity over the first year is seen, with a significant and progressive decline after 4 – 5 years on PD, especially relative to values at years 1 and 2. Significance was confirmed by the mixed-model analysis.

EFFECT OF BASELINE TRANSPORT CHARACTERISTICS

Baseline MTC Cr values by transport quartile were associated with different outcomes after 5 years on PD (Figure 3). Patients in the faster transport quartiles showed a tendency toward diminishing MTC Cr values, and patients in the slower quartiles tended to show increasing MTC Cr values over time. Those differences appeared just after year 1 and were most pronounced between the L and the H and HA quartiles, as shown by the post-hoc Bonferroni test: L vs H, $p = 0.001$, and L vs HA, $p = 0.003$.

To confirm those findings, we performed a mixed-model analysis to study factors influencing MTC Cr outcome. Taking baseline MTC Cr as a co-variable and time as the main factor, the model confirms that baseline MTC Cr values are associated with significantly different behavior only up to the end of the first year on PD ($p = 0.011$).

EFFECT OF PERITONITIS

During the study, 113 patients remained free from peritonitis, and 162 patients experienced 1 or more peritonitis episodes (195 episodes in total). Figure 4(A) shows how the appearance of peritonitis during year 1 was significantly associated with a neutralization of the decline in MTC Cr shown in patients free from peritonitis

during that period. However, in later annual periods, this inverse phenomenon was not repeated [Figure 4(B)]. Mixed-model and repeated measures analysis of the MTC Cr outcome confirmed the effect of early peritonitis, with minimal effects after year 4.

To determine whether the initial peritoneal transport type would condition the effect of peritonitis on MTC Cr outcome, we included baseline MTC Cr as a co-variable. The analysis confirmed the significant influence of peritonitis on MTC Cr outcome early during PD ($p < 0.023$) regardless of the starting MTC Cr value. Accumulated days of peritoneal inflammation showed an influence only during the first 3 years ($p = 0.005$, $p = 0.003$, and $p = 0.002$ respectively).

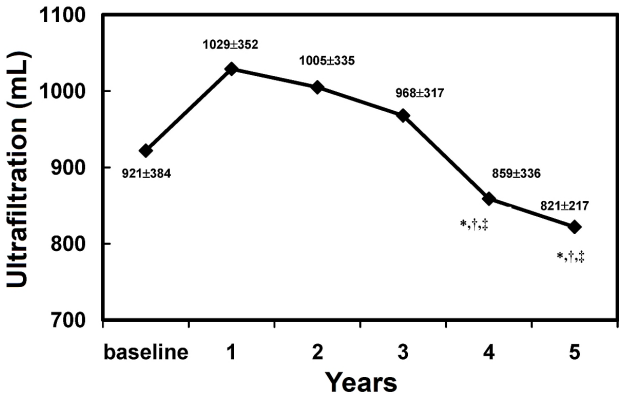


Figure 2 — Ultrafiltration capacity over time. Ultrafiltration capacity increased nonsignificantly during year 1. * Statistically significant decrease compared with year 1. † Statistically significant decrease compared with year 2. ‡ Statistically significant decrease compared with year 3.

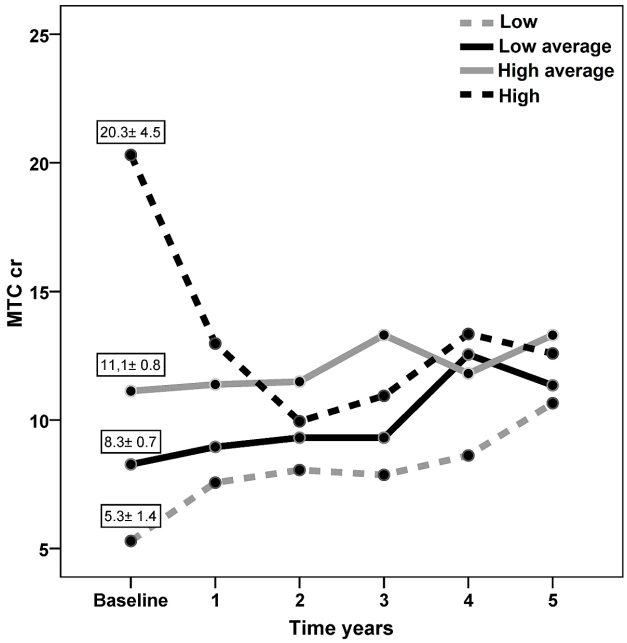
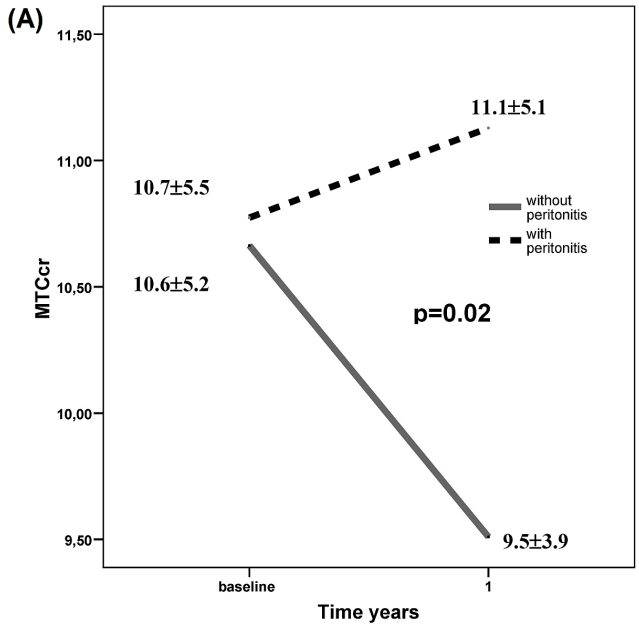


Figure 3 — Mass transfer coefficient of creatinine (MTCcr) during 5 years on peritoneal dialysis, by MTCcr quartile at baseline. Patients in the faster transport quartiles (high, high-average) tended to show a diminishing MTCcr; patients in the slowest transport quartile (low) tended to show an increasing MTCcr.

EFFECT OF HGE

The percentages of patients with HGE during years 1, 2, 3, 4, and 5 were 37.7%, 31%, 43.9%, 47.5%, and 52.2%



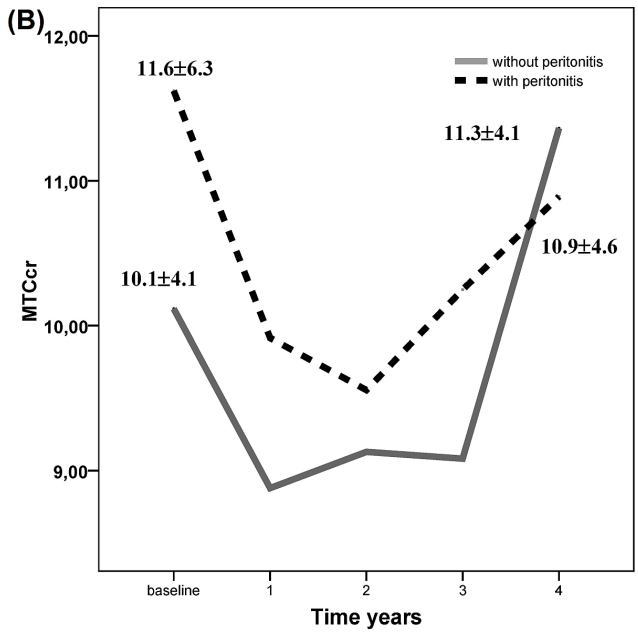


Figure 4 — (A) Evolution of the mass transfer coefficient of creatinine (MTCcr) during year 1 for patients with ($n = 185$) and without ($n = 89$) peritonitis. (B) Evolution of the MTCcr was not significantly different over a period of 4 years in patients with ($n = 48$) and without ($n = 20$) peritonitis.

respectively. Patients with HGE during year 1 showed a higher baseline MTC Cr value (11.7 ± 5.6 mL/min vs 10.0 ± 5.1 mL/min, $p = 0.012$) and a lower baseline UF capacity (803.4 ± 292.8 mL vs 943.5 ± 369.8 mL, $p < 0.001$), but no differences in RRF, age, or diabetes status.

Figure 5 shows the results for patients who reached 5 years on PD, in which a strong and statistically significant MTC Cr increase is noted for those with HGE; no changes were observed for patients having low glucose exposure (LGE).

Some of the HGE may be dictated by the basal transport type and the loss of RRF that occurs over time on PD. To avoid those interferences and to isolate the role of glucose exposure with regard to peritoneal function, we explored the effect of glucose in only the intermediate quartiles (HA, LA) of MTC Cr. Because overuse of glucose is less conditioned by the initial type of peritoneal transport in those patients, the expectation is that H transporters would massively overuse, and that L transporters would rarely overuse. As Figure 6 shows, in a group of patients starting with similar values for peritoneal function, overuse of glucose led to a significant divergence in the MTC Cr outcome during year 1.

With regard to RRF, we found no differences at baseline and at year 1 between patients with HGE and LGE (baseline: 3.9 ± 2.8 mL/min vs 4.1 ± 2.8 mL/min; year 1: 2.5 ± 2.2 mL/min vs 2.2 ± 1.8 mL/min respectively; all nonsignificant). At baseline, UF capacity was not different between those groups (924 ± 379 mL vs 824 ± 284 mL, nonsignificant). However, after the first year, patients with LGE have a higher UF than do patients with HGE (999 ± 313 mL vs 847 ± 290 mL, $p < 0.01$). No differences in days of active peritonitis during the first year were observed between the groups.

APD AND ICODEXTRIN USE

The analysis that follows is limited because, from the beginning of PD, only 21 and 14 patients started with icodextrin and APD respectively. In both groups, we found a nonsignificant trend toward a higher MTC Cr at baseline and a greater decline over year 1. The assessment of the influence of both factors was completed by mixed-model analysis, taking into account patients who started on icodextrin and APD over time. That analysis revealed that APD was not an influencing variable. By contrast, icodextrin use showed a statistically significant

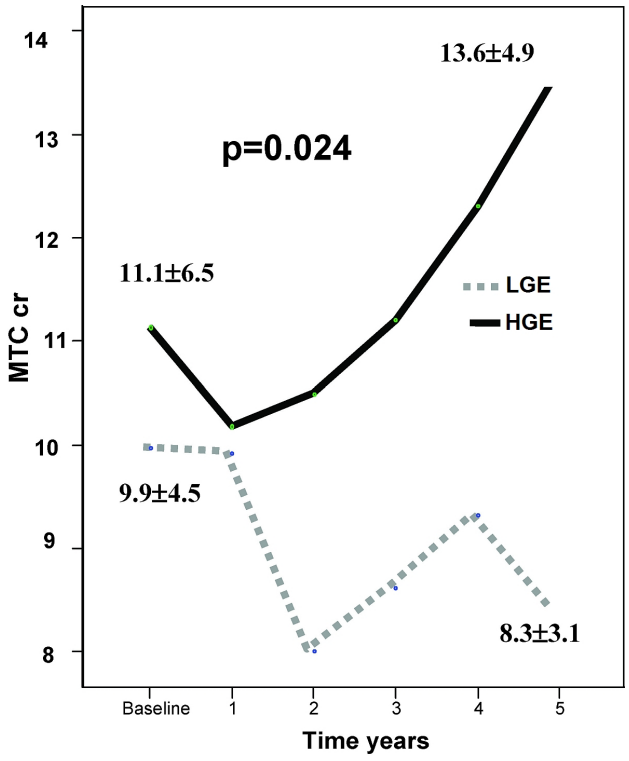


Figure 5 — In 12 patients with low glucose exposure (LGE), the mass transfer coefficient of creatinine (MTCcr) remained stable over time, but in 26 patients with high glucose exposure (HGE), the MTCcr showed an increase at some point in time. This difference was statistically significant ($p = 0.024$).

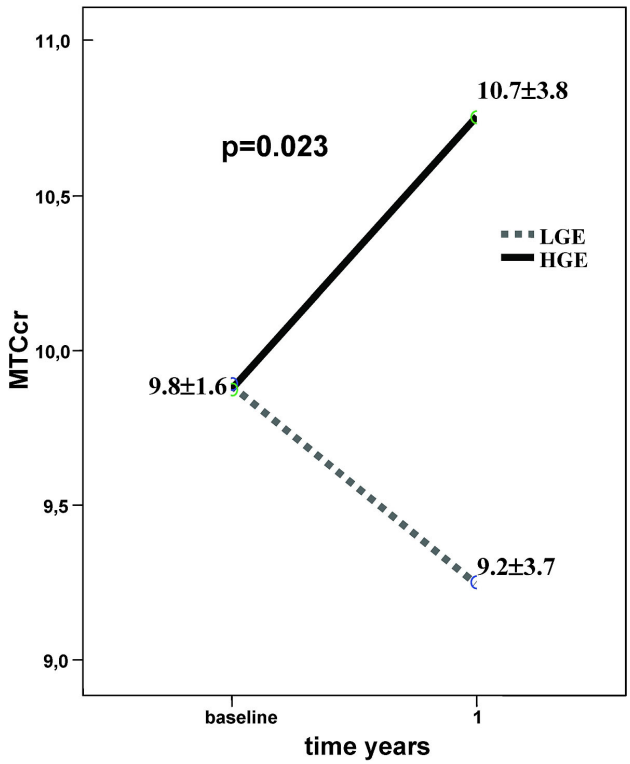


Figure 6 — Among patients in the intermediate transport quartiles (quartiles 2 and 3), those with high glucose exposure (HGE) in year 1 showed an increase in MTCcr. In those with low glucose exposure (LGE), the MTCcr diminished during year 1. This difference was statistically significant ($p = 0.023$).

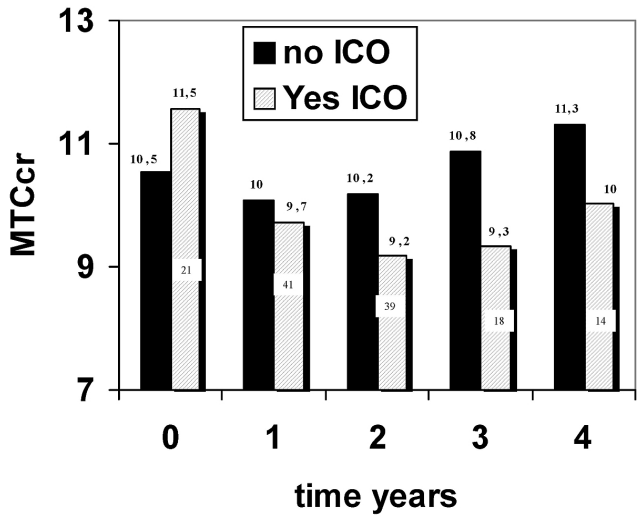


Figure 7 — The mixed model shows that, in patients using icodextrin (ICO), the mass transfer coefficient of creatinine (MTCcr) was lower in the subsequent year, although MTCcr values in those patients had been higher at baseline. During years 2 and 3, patients on ICO showed a significant decrease in MTCcr relative to baseline.

association with a lesser increase in MTC Cr over time, specifically when the baseline MTC Cr value was included in the model ($p = 0.041$, time–icodextrin–baseline MTC Cr). We then extended the analysis to explore changes in MTC Cr according to the use of icodextrin in the preceding year. Figure 7 presents the results of that mixed model, which shows that, in patients using icodextrin, MTC Cr was lower in the subsequent year, although it had been higher at baseline. The data are insufficient for analyses during later periods.

VINTAGE EFFECT

In the 1990s, we progressively introduced the use of APD (starting in 1995) and icodextrin (starting in 1998), and we changed the PD fluid used during peritoneal kinetic studies to 2.27% from 1.36% glucose. An analysis by vintage was therefore necessary. The MTC Cr and UF capacity outcomes in patients treated preferentially or totally during the 1980s and 1990s showed nonsignificant or non–clinically relevant differences.

DISCUSSION

In the present study, we tried to determine the influence of baseline peritoneal conditions on the development of FT status and, at the same time, to analyze how the other risk factors (the degree and timing of peritonitis and glucose exposure) modified that influence. With the present data, we confirm that initial FT and UF failure are reversible conditions that, under favorable conditions, tend to normalize over the first year on PD. As shown in Figure 3 and confirmed by the mixed-model analysis of peritoneal permeability, outcome at year 1 is not different for different transport values at baseline. That finding leads us to hypothesize that, with bioincompatible PD solutions, inherent and acquired FT are intrinsically and prognostically different. Attributing poorer survival and technique prognosis to transitory inherent FT seems to be inconsistent (14,21,22). Similarly, we suggest that the MTC Cr at year 1 is the most predictive value in the patient's history. For that reason, we emphasize the importance of analyzing the influence of peritonitis and HGE on peritoneal permeability during the first year.

We observed the influence of peritonitis from PD start: episodes occurring during year 1 invert the usual spontaneous trend of a reduction in solute transport. Cho *et al.* (23) proved that this behavior is associated with high levels of interleukin 6, transforming growth factor β, and vascular endothelial growth factor in peritoneal effluent. All those mediators cause peritoneal capillary vasodilation and FT, transitory and definitive. Episodes appearing over subsequent years also have an influence on peritoneal transport, leading to a switch to HD because of UF failure, as we previously described (24). Accumulated days of peritoneal inflammation and late peritonitis were the main causes of switches to HD and membrane failure in our population (data not shown), which might explain why we found no effect of peritonitis in the analysis from year 4 onwards. Somehow, patients that stay on PD beyond the fourth year are a selected population with a low incidence or influence of peritonitis.

Adverse effects from exposing the peritoneum to high glucose concentrations are abundantly observed with the use of bioincompatible PD fluids both in animal models (25,26) and in clinical studies (8,9). Animal models have shown the benefit of using fluids low in glucose degradation products (compared with conventional fluids) in terms of vasculopathy and fibrosis (27). We recognize that, in clinical studies, it is difficult to show the specific and separate effect of glucose content because of the generally unavoidable coincidence between high glucose use and peritoneal FT. To try to clarify that issue, we explored the influence of HGE on peritoneal transport only during year 1 in the group of patients with intermediate permeability. Choosing that group of patients and having ruled out differences in RRF, baseline UF, and peritonitis incidence, we were able to investigate the effects of glucose dose alone. In the selected group, a greater use of glucose was unquestionably associated with the maintenance of FT; patients who did not overuse

glucose showed a decline in solute permeability. Davies, writing alone and with colleagues (8,9), showed similar results, confirming that early overuse of glucose transforms the membrane in terms of transport. In our series, icodextrin consistently protected the peritoneum, given that the use of icodextrin was associated with a delay in the increase of solute permeability. Use of icodextrin from the very beginning of PD has also been associated with a greater decline in solute transport during year 1 among patients with FT at baseline (28). Moreover, icodextrin has been reported to stop the increase in peritoneal solute permeability in prevalent anuric patients on APD (29).

The main strengths of the present study are the significant number of patients being followed in a single unit using a stable peritoneal function methodology. Also, as shown in Figure 1(A), peritoneal function across time was similar in all patients (MTC Cr decline during the first 2 years). Finally, as shown in Table 1, the demographic and peritoneal transport characteristics of the 403 initial patients, the 275 patients studied repeatedly, and the 38 patients who reached 5 years on PD were all similar. Both findings suggest that these two analyzed PD populations (275 and 38 respectively) are representative of the entire population, leading us to think that the analysis excludes biased dropout or positive selection attributable to peritoneal membrane problems.

The main limitations of the present study are these:

- The change made in the functional evaluation of the peritoneum. The MTC Cr was determined using 1.36% glucose until 1990 and 2.27% glucose thereafter—although we previously showed that 1.36% and 2.27% glucose produced similar results (data not shown).
- The fact that, until 2004, no internationally accepted method to measure the UF rate was available (20). We had to use a clinically useful and reproducible 24-hour standard UF methodology that could, in fact, depend on factors other than pure peritoneal UF capacity—including RRF or an intake-driven need for volume removal. To rule out decisive influences on results, we analyzed the effect of 1980s and 1990s dialysis vintage on the transport parameter outcome, finding no significant differences.

In our population, membrane failure as the cause of technique failure was infrequent (occurring in only 16 patients). That circumstance might explain why some factors that usually show a negative influence with time (such as peritonitis and HGE) did not reach statistical significance in the Cox analysis for membrane failure.

CONCLUSIONS

Prognosis for the peritoneal membrane is independent of baseline transport characteristics. Fast transport and UF failure are reversible conditions when peritonitis and HGE are avoided during the early dialysis period. Year 1 is among the main determining factors of the membrane's future, with the MTC Cr at 12 months being the best predictor of future peritoneal function. Icodextrin helps with glucose avoidance and is associated with peritoneal protection. We think that these data provide a historical framework within which to compare the natural history of the peritoneal membrane under bioincompatible and biocompatible solutions.

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DISCLOSURES

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Resultados relacionados con el tratamiento con soluciones de diálisis más biocompatibles:

Capítulo 6. Función peritoneal durante los primeros años en diálisis peritoneal

6.2.1.1.- Estudio prospectivo de la evolución de la función peritoneal en pacientes tratados con soluciones más biocompatibles. Análisis comparativo con los pacientes tratados con soluciones convencionales

Este trabajo responde al objetivo 4

Las soluciones más biocompatibles han demostrado en estudios *in vitro* y *ex vivo* sus efectos beneficiosos sobre la membrana peritoneal (1,2). Sin embargo, su impacto sobre el transporte de pequeños solutos y agua no está aún aclarado.

El principal objetivo del presente estudio es conocer cuál es la evolución de la función peritoneal en los pacientes tratados con soluciones más biocompatibles, y cuáles son los factores que la determinan. Otro propósito ha sido comparar dicha evolución con la de los pacientes tratados con soluciones convencionales.

Pacientes y métodos

Se trata de un estudio prospectivo longitudinal, observacional, de cohortes, que incluye todos los pacientes que inician DP en el Hospital La Universitario La Paz desde el año 2004 hasta el 31 de mayo de 2013, tratados exclusivamente

con soluciones biocompatibles, y con al menos un estudio cinético peritoneal basal. Se excluyen los pacientes con historia previa de DP, los tratados con soluciones convencionales y los que presentaron peritonitis previa al estudio cinético basal.

Las soluciones biocompatibles de DP empleadas incluyen: soluciones glucosadas de pH neutro y baja concentración de PDGs: Physioneal (Baxter Healthcare Corporation®, Deerfield, IL, USA), Gambrosol Trio (Gambro Lundia AB®, Lund, Sweden), Bicavera y Balance (Fresenius Medical Care®, Bad Homburg, Germany).

Para evaluar la evolución de la función peritoneal a lo largo del tiempo, se seleccionaron los pacientes que tenían al menos un estudio cinético peritoneal basal y otro a los 12 meses (n= 74). Este grupo se comparó con un grupo histórico de pacientes tratados exclusivamente con soluciones convencionales (n= 275) que iniciaron DP en nuestra unidad entre 1980 y 2002, y que había sido analizado previamente por nuestro grupo con la misma metodología (3). Las soluciones convencionales utilizadas fueron: Stay-Safe (Fresenius Medical Care®, Bad Homburg, Germany) y Dianeal (Baxter Healthcare Corporation®, Deerfield, IL, USA).

Se registraron al inicio de DP los siguientes parámetros: edad, sexo, causa de la enfermedad renal, modalidad de DP (DPA o DPCA) y presencia o no de Diabetes mellitus. Además se recogieron anualmente (a los 12, 24 y 36 meses del inicio de diálisis) los siguientes datos: antecedente de peritonitis, número de episodios y días de inflamación peritoneal, cambios de modalidad de DP (DPA o DPCA), uso de soluciones glucosadas y no glucosadas. Las soluciones no glucosadas incluyen: icodextrina al 7,5% (Extraneal [Baxter Healthcare

Corporation®, Deerfield, IL, USA]) y Nutrineal al 1,1% (Baxter Healthcare Corporation®, Deerfield, IL, USA).

Tanto basalmente como a los 12, 24 y 36 meses de seguimiento, se registraron los siguientes parámetros: función renal residual (FRR), proteína C reactiva (PCR), cociente dializado/plasma de creatinina (D/P-Cr), coeficiente de transferencia de masas de creatinina (MTC-Cr) y urea (MTC-U), capacidad de ultrafiltración (UF/4h) y cribado de sodio.

Las causas de fin del seguimiento fueron: muerte del paciente, transferencia a HD o trasplante, recuperación de la FRR o traslado de centro.

Análisis Estadístico:

El programa estadístico utilizado fue el SPSS software (Versión 15.0, Chicago, IL, EE.UU). Se consideró estadísticamente significativo un valor de $p<0.05$. Los datos se expresan como media ± desviación estándar para variables cuantitativas de distribución normal, y como mediana + rango intercuartílico para variables no paramétricas. Las variables categóricas se expresan como proporciones y porcentajes. La comparación de proporciones se realizó con el test de χ^2 (Chi-cuadrado), las medias mediante la prueba de la t de Student y las medianas con el test de Mann-Whitney.

La distribución normal de los distintos parámetros de función peritoneal se analizó mediante la prueba de Kolmogorov-Smirnov. Para el análisis de correlaciones se utilizó el coeficiente de correlación de Pearson para variables paramétricas y de Spearman para las no paramétricas, respectivamente. La comparación de la evolución de los parámetros de función peritoneal en función del tiempo y del tipo de solución empleada, se realizó mediante el análisis de

regresión lineal para medidas repetidas o utilizando los modelos mixtos, según se compararan dos o más determinaciones en el tiempo. Para el análisis *post-hoc* se empleó el test de Bonferroni. Los resultados deben interpretarse de la siguiente forma:

• "grupo significativo": existen diferencias en función del tipo de solución de DP empleada por cada grupo, pero la variación en el tiempo es similar en ambos (se mantiene el paralelismo).

• "tiempo significativo": el tiempo afecta a ambos grupos de manera similar.

• "modelo significativo": la interacción grupo-tiempo es significativa ($p < 0.05$).

RESULTADOS

- **Características clínicas y parámetros de función peritoneal al inicio de DP**

En el estudio basal se evaluaron 140 pacientes tratados con soluciones biocompatibles (Tabla 1), con una mediana de edad de 52.22 años (rango 38.09-62.43). El 65% de los pacientes eran varones, un 27.1% diabéticos y las causas principales de enfermedad renal fueron: nefropatía diabética (20%), glomerulonefritis crónica (16.4%) y nefritis intersticial (15%).

Tabla 1: Características clínicas y funcionales de los pacientes incluidos en el estudio basal y al año

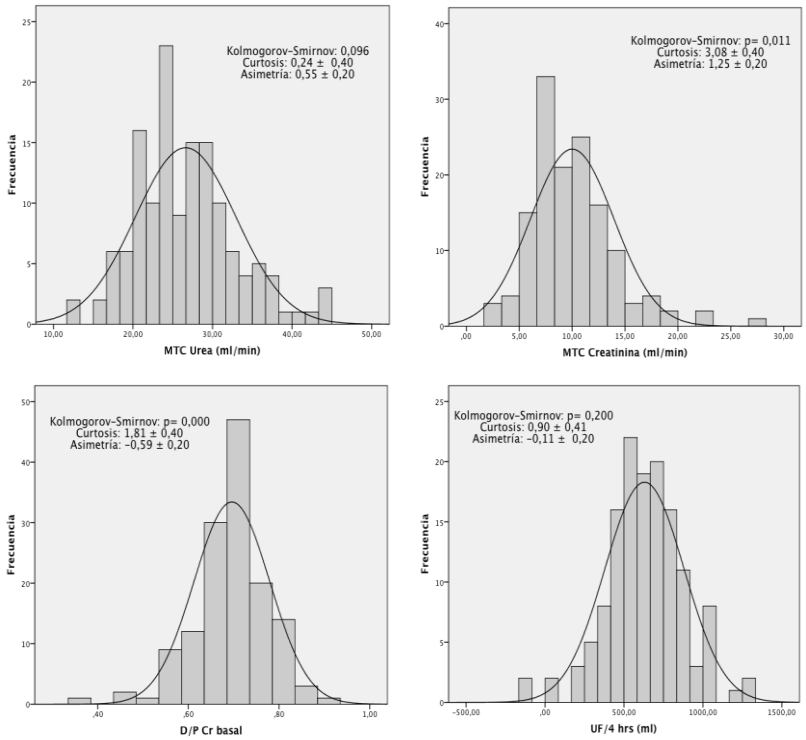
Variables[a]	Basal	Incluidos en el estudio al 1er año
Nº de pacientes	140	74
Edad (años)	50,21 ± 15,89	49,16 ± 15,74
Sexo femenino	49 (35%)	29 (39,2%)
DM	38 (27,1%)	25 (33,8%)
DPA	72 (51,4%)	35 (47,3%)
Icodextrina	64 (45,7%)	33 (44,6%)
Aminoácidos	34 (24,2%)	18 (24,3%)
SEG	10 (7%)	3 (4,1%)
MTC-U (ml/min)	26,71 ± 6,35	26,61 ± 7,26
MTC-Cr (ml/min)	10,0 ± 3,95	9,79 ± 3,45
Relación MTC U/Cr	2,97 ± 1,15	2,93 ± 0,98
UF/4 h (ml)	629,63 ± 250,79	598,31 ± 249,05
D/P-Cr	0,69 ± 0,08	0,69 ± 0,07
FRR (ml/min)	6,15 ± 3,39	6,27 ± 3,25
Cribado de sodio	3,10 ± 4,03	2,39 ± 4,39

Abreviaturas: DM: diabetes mellitus, DPA diálisis peritoneal automática, SEG: sobreexposición a glucosa, MTC-U: coeficiente de transferencia de masas de urea, MTC-Cr: coeficiente de transferencia de masas de creatinina, MTC U/Cr: relación entre el MTC urea y creatinina, UF: ultrafiltración, D/P-Cr: cociente dializado/plasma de creatinina, FRR: función renal residual.

[a] No se encontraron diferencias estadísticamente significativas entre ambos grupos

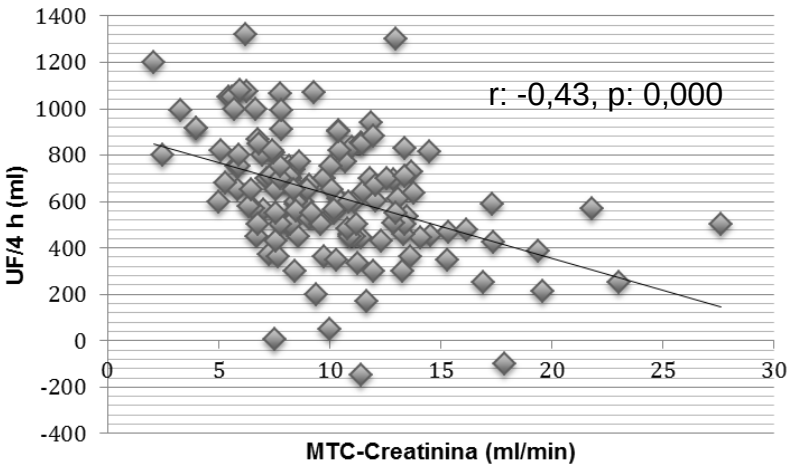
El MTC-U y la UF/4h presentaron una distribución normal, a diferencia del MTC-Cr y el D/P-Cr (Figura 1).

Figura 1: Distribución de los distintos parámetros de función peritoneal basales



Se encontró una correlación lineal inversa significativa entre el MTC-Cr y la capacidad de UF/4h (r= -0.43, p <0.000) (Figura 2).

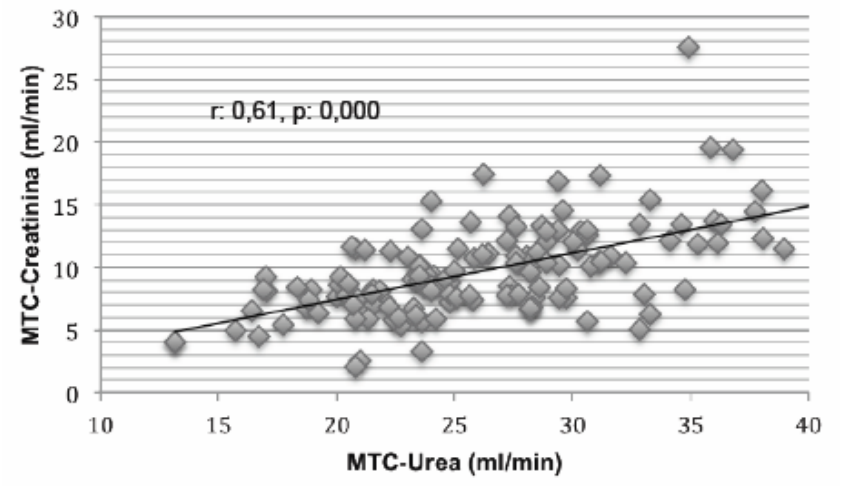
Figura 2: Análisis de correlación lineal entre MTC-Cr y UF/4h



El MTC-Cr basal se correlacionó positivamente con el MTC-U (r= 0.6; p=0,000) (Figura 3) y la PCR (r= 0.23; p=0.005), e inversamente con el cribado de sodio (r= -0.32; p=0.000). No se encontró correlación entre el MTC-Cr y otras variables clínicas, como la edad, sexo, diabetes y FRR.

Cuando se analizaron los subgrupos con valores extremos de UF y MTC-Cr (cuartiles 1 y 4), en el análisis multivariante (incluyendo edad, sexo, diabetes y otros parámetros de función peritoneal) no se encontraron factores independientes que explicaran esos valores extremos.

Figura 3: Análisis de correlación lineal entre el MTC-Cr y el MTC-U



- **Evolución del transporte peritoneal**

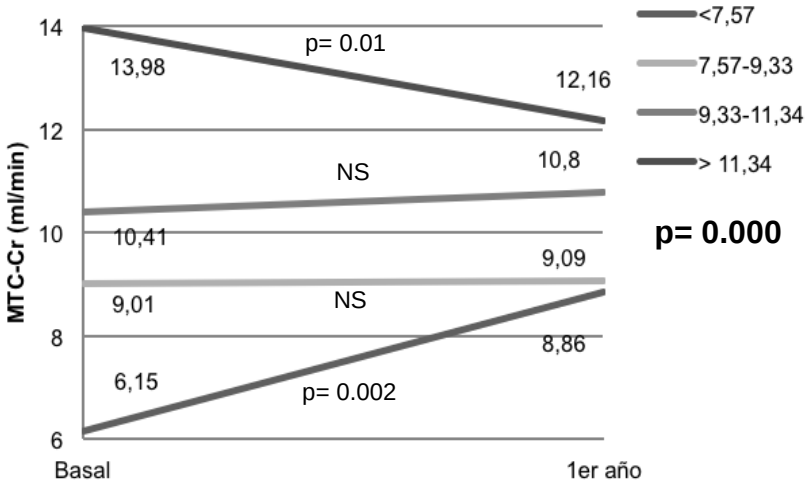
I. Evolución durante el primer año

Los parámetros de función peritoneal se analizaron al año en 74 pacientes tratados con soluciones biocompatibles. Las características clínicas y de función peritoneal basales fueron similares a las de los 140 pacientes del grupo basal (Tabla 1). Tras el primer año en DP, se observó disminución significativa de la FRR (6.27 ± 3.25 frente a 4.97 ± 3.54 ml/min, p=0.001) y aumento de la UF/4h (598.31 ± 249.0 frente a 658.91 ± 239.93 ml/4h, p=0.04). No se

apreciaron cambios en el resto de los parámetros de función peritoneal (MTC-Cr, MTC-U, D/P-Cr), ni hubo diferencias en función del sexo o de la presencia de diabetes.

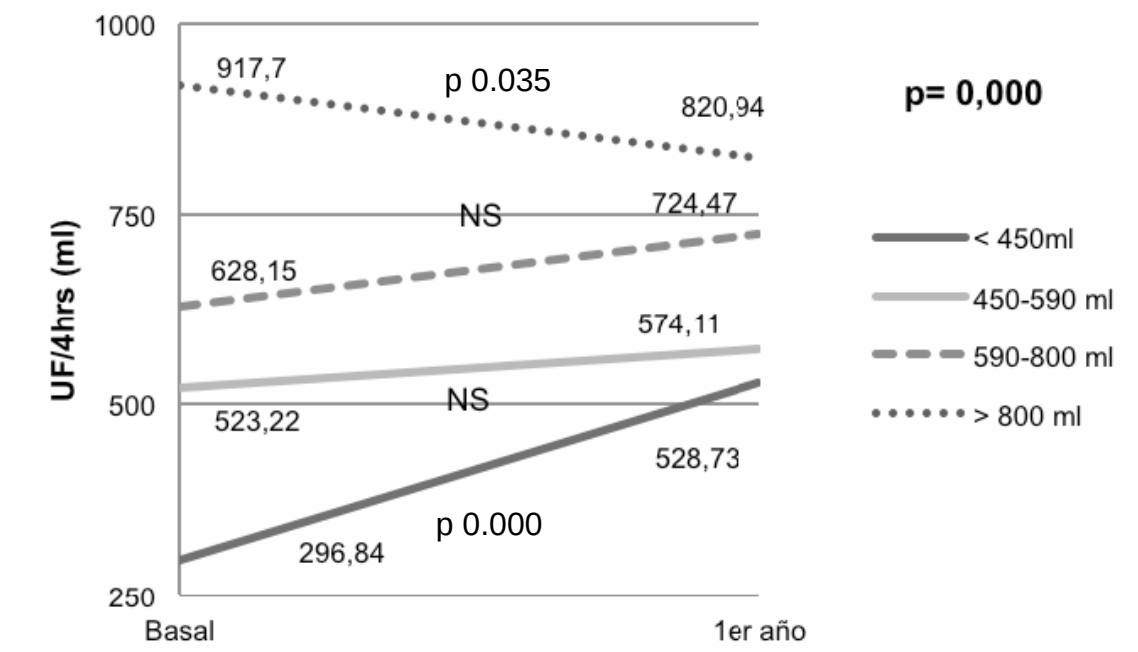
Con el fin de comparar la evolución de los parámetros de función peritoneal según el MTC-Cr basal, se establecieron 4 grupos de pacientes en función de la distribución por cuartiles del MTC-Cr basal: bajos transportadores (<7.57 ml/min, n = 19), bajo-promedio (7.57 – 9.32 ml/min, n = 18); alto-promedio (9.33 a 11.34 ml/min, n = 19), y altos transportadores (> 11.34 ml/min, n=17). Cuando se analizó la evolución del MTC-Cr en función del MTC-Cr basal (Figura 4), se observó un fenómeno de convergencia a la mediana, de modo que los pacientes con bajo transporte (primer cuartil), mostraron un aumento significativo de MTC-Cr (6.15 ± 1.21 vs 8.86 ± 2.7, p=0,002), mientras que los pacientes con alto transporte (cuarto cuartil) presentaron una disminución significativa (13.98 ± 2.25 vs 12.16 ± 2.55 ml/min, p= 0.01). Los pacientes con transporte bajo-promedio y alto-promedio (2º y 3º cuartiles) no mostraron cambios significativos.

Figura 4: Evolución del MTC-Cr al año según los cuartiles del MTC-Cr basal en el grupo de soluciones biocompatibles. En el análisis post-hoc hubo diferencias estadísticamente significativas cuando cada subgrupo se comparó con el resto.



También se establecieron cuartiles en función de la capacidad de UF basal en los pacientes incluidos en el estudio al año: <450 ml/min (n= 19), 450-589ml (n= 18); 590-800ml (n= 19), y > 800ml (n= 17). Al analizar la evolución de la UF en función del cuartil de UF de partida, se observó un aumento significativo de la UF en el 1er cuartil (296.84 vs 528.73; p=0.000), una tendencia no significativa al aumento de UF en el 2º y 3º cuartil, y un descenso significativo de la UF en el 4º cuartil (917.7 vs 820.94; p=0.035)(Figura 5).

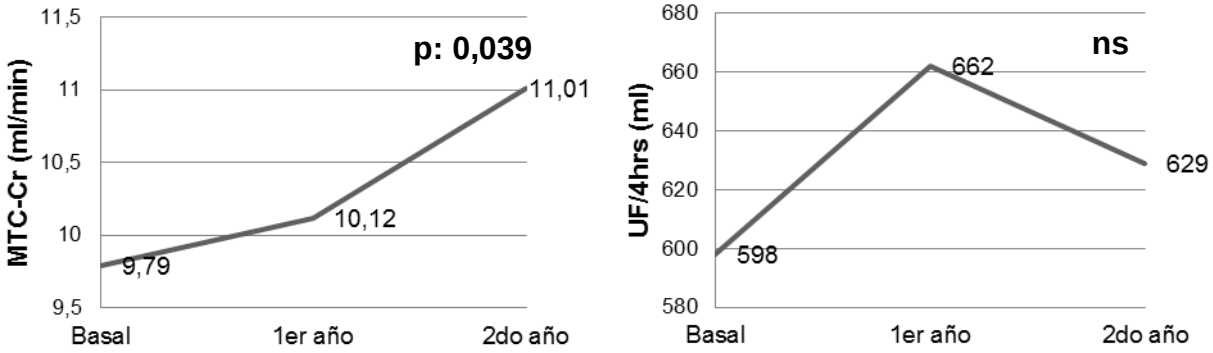
Figura 5: Evolución de la UF/4h al año según los cuartiles de la UF basal en el grupo de soluciones biocompatibles. El análisis post-hoc de la UF mostró diferencias estadísticamente significativas para 1º y 4º cuartiles.



II. Evolución a largo plazo

Los datos de función peritoneal del 2º año se registraron en 42 pacientes y del 3º año en 15 pacientes. Los pacientes mostraron una disminución progresiva y significativa de la FRR (p=0.000). El MTC-Cr aumentó durante el seguimiento, sobre todo en el segundo año (p=0.039)(Figura 6), el MTC-U no mostró variaciones significativas y el D/P-Cr aumentó de forma significativa (p=0.023). La UF/4h aumentó de forma significativa durante el primer año, disminuyendo en el segundo año y estabilizándose posteriormente (estas variaciones en el tiempo en el análisis de los modelos mixtos no fueron significativas)(Figura 6).

Figura 6. Evolución a largo plazo de la UF/4h y el MTC-Cr en los pacientes tratados con soluciones biocompatibles



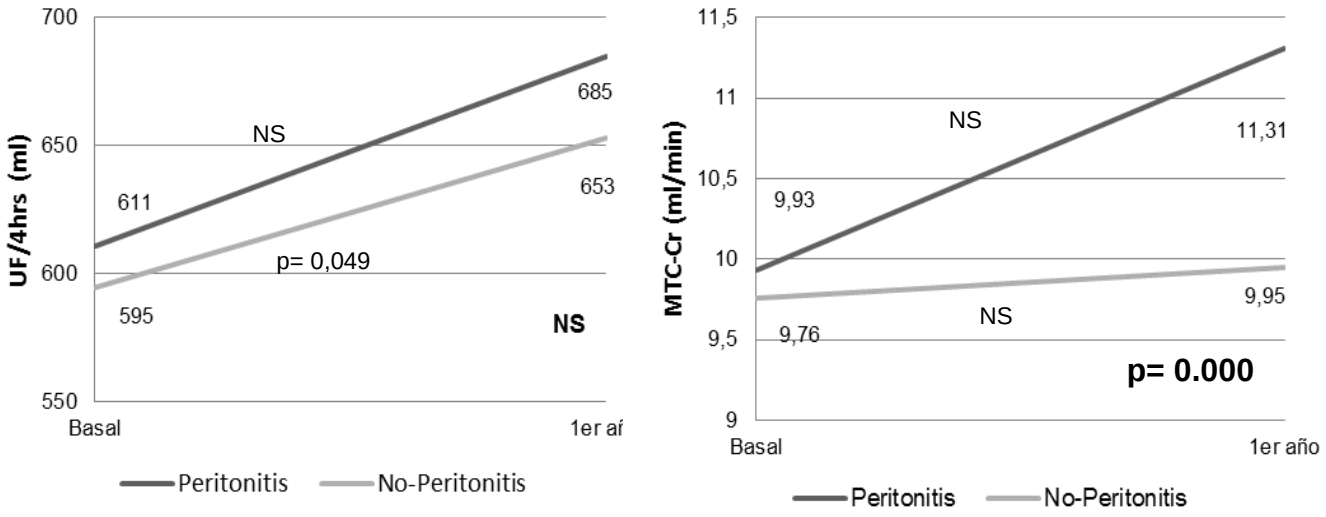
III. Efecto de las peritonitis sobre la función peritoneal

Durante el estudio, 56 pacientes no presentaron ningún episodio de peritonitis, mientras que 18 pacientes sufrieron uno o más episodios (24 episodios en total). Trece pacientes tuvieron al menos un episodio de peritonitis en el primer año.

Durante el primer año (Figura 7), se observó un aumento de la capacidad de UF, que fue significativo en los pacientes sin peritonitis (595.4 ± 260.5 frente a

653.2 ± 194.8 ml/4h, p=0.04), pero no en el grupo con peritonitis (611.53 ± 195 frente a 685 ± 397 ml/4h, p=0.17) Con respecto al transporte de pequeños solutos, los pacientes con peritonitis presentaron un aumento del MTC-Cr en el primer año con respecto a los pacientes sin peritonitis.

Figura 7: Evolución de la UF/4h y el MTC-Cr durante el primer año en los pacientes con o sin antecedente de peritonitis. La comparación de las pendientes entre los dos grupos, no mostró diferencias significativas en la UF/4h, pero sí en el MTC-Cr ($p<0.05$).



IV. Efecto de la exposición a soluciones glucosadas sobre la función peritoneal

Durante el 1º, 2º y 3º año, el porcentaje de pacientes con sobreexposición a glucosa fue de 6.7%, 9.3% y 26.6%, respectivamente. Cuando se analizaron los efectos de la exposición a soluciones glucosadas del 2.27% (21 pacientes, 28%) frente al uso exclusivo de soluciones al 1.36% (53 pacientes, 70.7%) sobre la evolución de los parámetros de función peritoneal (MTC-Cr, MTC-U,

D/P-Cr y UF/4h), no se encontraron diferencias significativas en el tiempo (datos no mostrados).

- **Comparación entre los pacientes con soluciones biocompatibles y convencionales: Características basales**

En la Tabla 2 se detallan las diferencias entre ambos grupos. Los pacientes con soluciones biocompatibles utilizaban con más frecuencia DPA, icodextrina y soluciones con aminoácidos, mientras que el grupo de pacientes con soluciones convencionales mostró de forma significativa una mayor exposición a la glucosa. Por lo que respecta a los parámetros de función peritoneal basales, el grupo de soluciones convencionales presentó una mayor UF basal, menor MTC-U y menor FRR. El MTC-Cr basal en el grupo de soluciones convencionales es mayor que en el grupo de biocompatibles, aunque sin diferencias estadísticamente significativas. Cuando se compararon los cuartiles del MTC-Cr de ambos grupos, se observó que el 3º y 4º cuartiles fueron significativamente más altos en el grupo de las soluciones convencionales (11.26 ± 0.89 frente a 10.47 ± 0,48; p=0.000, y 17.85 ± 5.29 frente a 14.42 ± 2.85, p=0.01; respectivamente).

En el análisis multivariante, el MTC-Cr basal elevado (p=0.000) y el uso de soluciones convencionales (p=0.009) fueron factores independientes de una menor UF basal.

Tabla 2. Características clínicas basales de los pacientes tratados con soluciones biocompatibles y convencionales

	Soluciones biocompatibles (n=74)	Soluciones convencionales (n=275)
Edad (años)	49,16 ± 15,74	52,58 ± 15,45
Tiempo en DP (meses)	25,39 (12,7-69,7)	29,91 (12-190)
Sexo femenino	29 (39,2%)	136 (49,5%)
DM	25 (33,8%)	63 (22,9%)
DPA	35 (47,3%)[a]	14 (5,1%)[a]
Icodextrina	33 (44,6%)[a]	21 (7,6%)[a]
Aminoácidos	18 (24,3%)[a]	0[a]
SEG	3 (4,1%)[a]	86 (31,3%)[a]

Abreviaturas: DP: diálisis peritoneal, DM: diabetes mellitus, DPA diálisis peritoneal automática, SEG: sobreexposición a glucosa, [a] p<0,05

- **Comparación en la evolución de la función peritoneal entre ambos grupos (soluciones biocompatibles o convencionales)**

Al comparar la evolución durante el 1º, 2º y 3º año en ambos grupos (Tabla 3), persistían las diferencias en cuanto al mayor uso de DPA, icodextrina y soluciones de aminoácidos y menor exposición a glucosa en el grupo de soluciones biocompatibles, sin diferencias en cuanto a las peritonitis.

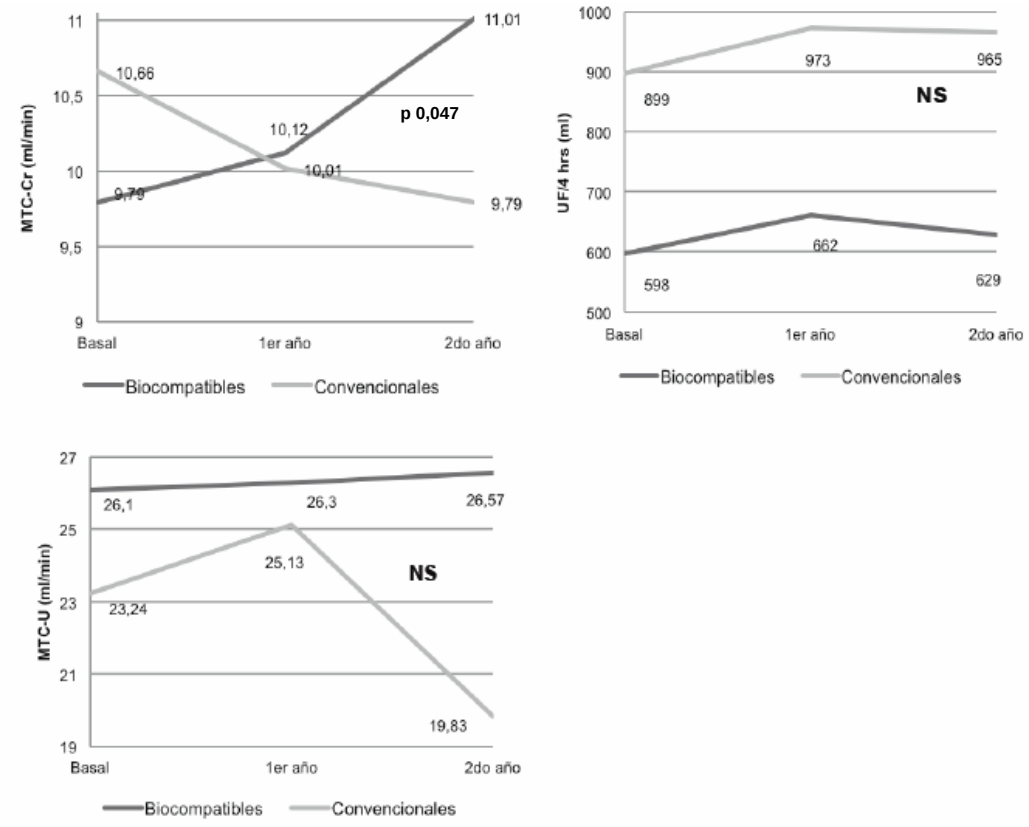
Se observó un incremento progresivo significativo del MTC-Cr en el grupo de soluciones biocompatibles en comparación con el grupo convencional (p=0.047)(Figura 8). El MTC-U no varió significativamente en el tiempo ni al comparar ambos grupos. La UF fue significativamente mayor en el grupo de pacientes tratados con soluciones convencionales durante todo el seguimiento (basal, 1º y 2º año). Su evolución en el tiempo fue similar en ambos grupos.

Tabla 3: Evolución de los parámetros de función peritoneal en los pacientes tratados con soluciones biocompatibles y convencionales

	Basal		Primer año		Segundo año	
	Biocompatibles (n= 74)	Convencionales (n= 275)	Biocompatibles (n= 74)	Convencionales (n=275)	Biocompatibles (n= 43)	Convencionales (n= 170)
MTC-Cr (ml/min)	9,79 ± 3,45	10,68 ± 5,33	10,12 ± 3,08	10,01 ± 4,43	11.09 ± 5.62	9.79 ± 3.87
MTC-U (ml/min)	26,61 ± 7,26 [a]	23,24 ± 7,94 [a]	26,3 ± 7,05	25,13 ± 7,26	26,57 ± 7,26 [a]	19,83 ± 5,57 [a]
Relación MTC U/Cr	2,93 ± 0,98 [a]	2,42 ± 0,86 [a]	2,75 ± 0,79	2,94 ± 1,51	2,6 ± 0,66 [a]	2,23 ± 0,86 [a]
UF/4h (ml)	598,3 ± 249,05 [a]	899,1 ± 357,5 [a]	662,5±240,2 [a]	973,3 ± 342,3 [a]	629,7 ± 227,3[a]	965,9 ± 367,5 [a]
FRR (ml/min)	6,27 ± 3,25 [a]	4,04 ± 2,77 [a]	4,97 ± 3,54 [a]	2,6 ± 2,4 [a]	3,2 ± 2,68 [a]	0 ± 0 [a]

Abreviaturas: MTC-U: coeficiente de transferencia de masas de urea, MTC-Cr: coeficiente de transferencia de masas de creatinina, MTC U/Cr: relación entre el MTC urea y creatinina, UF: ultrafiltración, FRR: función renal residual, [a] p<0,05

Figura 8: Evolución del MTC-Cr y la UF hasta el 2º año en los pacientes tratados con soluciones biocompatibles y convencionales



Por último, cuando analizamos la correlación entre UF y MTC-Cr en ambos grupos, observamos que el grupo de soluciones convencionales al inicio de DP muestra una muy débil correlación inversa entre ambos (r: -0.18, p=0.0003), que aumenta progresivamente al año (r: -0.21; p=0.000) y a los dos años (r:-0.46, p=0.000). En el grupo de soluciones biocompatibles, esta relación se mantiene inalterada con el paso del tiempo (r: -0.37, p=0.001 basal; r: -0.34, p=0.000 al 1º año, y r: -0.35, p=0.021 a los dos años).

Discusión

Los productos de degradación de la glucosa (PDGs) que contienen las soluciones convencionales de DP lesionan el mesotelio y reducen su capacidad de regeneración, dando lugar a la formación de productos de glicosilación avanzada (AGEs) y activación de sus receptores. Éstos se depositan en el peritoneo, iniciando una señal pro-inflamatoria que provoca un estado inflamatorio crónico, con pérdida de la monocapa mesotelial, fibrosis y aumento de la microvasculatura peritoneal (4). El conocimiento de los efectos nocivos de las soluciones convencionales de DP ha provocado que en los últimos 15 años se hayan desarrollado nuevas soluciones más biocompatibles, con un pH neutro y baja concentración de PDGs. Estas soluciones han mostrado, tanto en estudios *in vitro* como ex *vivo*, su capacidad para suprimir la mayor parte de los efectos adversos de las soluciones convencionales sobre la membrana peritoneal (1,2).

En el presente trabajo, hemos analizado la evolución de la función peritoneal en los pacientes tratados en nuestra unidad de DP con estas nuevas

soluciones, comparándola con los pacientes tratados con soluciones convencionales.

Evaluación de la función peritoneal basal

Varios estudios han demostrado que la función peritoneal al inicio de la DP es muy variable (5), hecho que ha sido confirmado en el presente trabajo. El transporte de pequeños solutos, representado por el MTC-Cr y MTC-U, presenta una fuerte correlación, así como el MTC-Cr y la UF, y esta correlación se mantiene estable en el tiempo. En base a los hallazgos del presente estudio, podemos afirmar que existen pequeñas diferencias en el comportamiento del transporte peritoneal en función del tipo de solución de diálisis empleada. En los pacientes tratados con soluciones convencionales, al inicio de DP existe una correlación inversa entre el MTC-Cr y la UF/4h (r: -0.18), relación que a lo largo del tiempo se hace más fuerte, probablemente por un proceso de adaptación de la membrana peritoneal. Aun así, los datos comparativos del estudio de la función peritoneal basal en ambos grupos confirman que la variabilidad funcional peritoneal inherente definitivamente existe.

Los factores que determinan la variabilidad del transporte peritoneal no están aclarados, habiéndose relacionado con la edad, el sexo y la superficie corporal (6). En nuestro estudio, los principales factores que determinan la UF al inicio de DP son el transporte de pequeños solutos y el cribado de sodio. Además, hemos observado que el MTC-Cr basal se correlacionó con la PCR, pero no con la edad, sexo, presencia de diabetes ni FRR. La asociación del transporte de pequeños solutos con la PCR ha sido previamente sugerida (7) y apoya la hipótesis de que la inflamación sistémica, sería un reflejo de los cambios que ocurren en la vasculatura peritoneal, tales como la vasodilatación y

neoangiogénesis, que serían en parte responsables de las alteraciones del transporte peritoneal (8).

Los factores genéticos, entre ellos polimorfismos en el gen de la IL-6, han sido postulados como posibles favorecedores de la variabilidad del transporte peritoneal inicial de solutos y agua.

Evolución de la función peritoneal con soluciones biocompatibles: comparación con las soluciones convencionales

Los pacientes tratados con soluciones biocompatibles muestran un aumento progresivo del transporte de pequeños solutos (MTC-Cr) a lo largo del tiempo. Sin embargo, su evolución durante el primer año depende del MTC-Cr de partida, de forma que los AT inherentes tienden a disminuir el transporte, y los bajos transportadores a aumentarlo. Este fenómeno de convergencia a la mediana fue ya previamente observado en pacientes tratados con soluciones convencionales (3) y probablemente responde a un proceso adaptativo del peritoneo tras sus primeros contactos con el líquido de diálisis. Este fenómeno de aumento del MTC-Cr en el primer año en DP observado en los pacientes tratados con soluciones biocompatibles, no se aprecia en los pacientes tratados con soluciones convencionales.

Los estudios comparativos de la evolución de la función peritoneal con ambos tipos de soluciones muestran resultados contradictorios, probablemente debido a las diferencias en las poblaciones de pacientes seleccionadas, al diseño y la metodología empleados. Algunos estudios no han encontrado diferencias en el transporte de solutos entre ambos grupos (9,10,11), mientras que otros estudios han observado mayor transporte de solutos con soluciones bajas en PDGs (mayor D/P-Cr) [12,13,14].

Con respecto a la evolución de la UF, en los pacientes tratados con soluciones biocompatibles existe un aumento significativo durante el primer año, siendo mayor el aumento en los pacientes con menor UF basal. Es de destacar que los pacientes tratados con soluciones convencionales mostraban desde el inicio una mayor UF, hecho que ya ha sido observado por otros autores (12,13,14). Sin embargo, la interpretación de los resultados de los distintos estudios es complicada, dado que no existe uniformidad en cuanto al uso de soluciones empleadas, métodos para evaluar la UF (UF diaria, UF/4h del estudio cinético…).

Lo que está demostrado es que el uso agudo de soluciones con bajo contenido en PDGs y lactato/bicarbonato como buffer durante el PET, comparado con el uso de soluciones convencionales con lactato, no parece tener influencia sobre los parámetros de transporte de pequeños solutos, la absorción de la glucosa y la UF (15,16). Varios estudios (10,13), entre ellos el nuestro, sugieren que el tratamiento prolongado con soluciones biocompatibles se asocia a una menor tasa de UF.

Como ya hemos comentado, a largo plazo el uso de soluciones biocompatibles se asocia a un aumento del transporte de pequeños solutos y descenso del transporte de agua (UF). Este fenómeno ha sido confirmado en varios estudios (12,13,14), como el Eurobalance, con un diseño prospectivo cruzado, que demostró cómo el paso de soluciones convencionales a biocompatibles se asoció a una disminución de la UF y aumento del D/P-Cr, mientras que el paso contrario conllevó un aumento de la UF y disminución del D/P-Cr. Varios autores han observado (14) un D/P-Cr significativamente más elevado y una

UF/4h inferior desde el inicio de DP en los pacientes tratados con soluciones bajas en PDGs (12).

Todos estos estudios sugieren que según el contenido en PDGs de la solución de DP empleada, podría ejercer efectos diferentes a nivel de la vasculatura peritoneal que se traducen en el desarrollo de una mayor permeabilidad a solutos en el caso de las soluciones bajas en PDGs. Es importante resaltar que el aumento progresivo del transporte de solutos observado en los pacientes tratados con soluciones bajas en PDGs no se acompaña de déficit de UF, lo que cuestiona el paradigma de la relación inversa entre el transporte de agua y solutos ampliamente conocida y descrita con las soluciones convencionales. Las razones que podrían explicar estas diferencias en el transporte de agua y pequeños solutos con las soluciones biocompatibles no están claras, aunque existen algunas hipótesis. En estudios animales, se ha observado que el uso de soluciones ricas en PDGs produce un aumento de la proliferación microvascular y fibrosis submesotelial, lesiones no observadas con el uso de soluciones biocompatibles (17). Además, el cambio de soluciones convencionales a biocompatibles en ratas se asoció a recuperación de la capacidad de UF, disminución de la angiogénesis y menor inducción de fibrosis (18).

Por otro lado, algunos estudios sugieren que el agente utilizado como buffer puede provocar cambios en la tasa de UF. Es conocido que concentraciones altas de lactato pueden provocar vasodilatación (19), lo que a nivel peritoneal se traduciría en una mayor permeabilidad de la membrana y en una reducción de la capacidad de UF peritoneal. Por su parte, concentraciones elevadas de bicarbonato pueden generar gran cantidad de pCO2 extracelular, que al

difundirse al interior de la célula causaría acidosis y secundariamente vasodilatación en la microcirculación, de forma que sus efectos serían similares a los observados con altas concentraciones de lactato.

En nuestro estudio, debido a la utilización de diferentes tipos de buffer en las soluciones bajas en PDGs, es difícil determinar si pueden afectar de algún modo la UF.

En resumen, los estudios que han analizado el impacto a corto plazo de las nuevas soluciones biocompatibles sobre el transporte de pequeños solutos y la capacidad de UF son contradictorios, por lo que se requieren nuevos estudios controlados para aclarar este aspecto.

Efectos de la exposición a la glucosa

En el grupo histórico tratado con soluciones convencionales, la sobreexposición a glucosa fue mucho más prevalente (31.3%) que en el grupo de soluciones biocompatibles (4.1%). Esto es debido probablemente a la introducción de nuevas soluciones, como la icodextrina, y al aumento del uso de la DPA frente a la DPCA, que han permitido conseguir un control más adecuado de la volemia sin necesidad de la sobreutilización de soluciones hipertónicas. Esto se demuestra por el mayor porcentaje de pacientes tratados con icodextrina y DPA en el grupo de soluciones biocompatibles al compararlo con el grupo de convencionales (44.6% vs 7.6%, 47.3% vs 5.1%; respectivamente). En nuestro estudio, no observamos diferencias en la evolución de la función peritoneal en función del uso de soluciones hipertónicas o soluciones al 1.36%, probablemente por el bajo uso de soluciones glucosadas hipertónicas y por el corto seguimiento. Como ha sido observado por estudios previos (5), la exposición temprana a grandes cantidades de glucosa suele preceder en el

tiempo a las alteraciones funcionales del peritoneo que presentan algunos pacientes en DP a largo plazo.

Efectos de la peritonitis sobre la función peritoneal

La peritonitis es el principal factor que influye sobre la evolución de la función peritoneal durante el primer año, ejerciendo efectos nocivos tanto en el transporte de agua como en el de pequeños solutos (20). En nuestra cohorte de pacientes, la presencia de peritonitis durante el primer año provoca un aumento de la permeabilidad a pequeños solutos (MTC-Cr), hecho no observado en pacientes sin peritonitis. Además, los pacientes con peritonitis muestran un escaso aumento de la UF en contraste con los pacientes sin peritonitis, que presentan un aumento significativo de la UF al final del primer año. Estos resultados son similares a los observados previamente en pacientes tratados con soluciones convencionales (3,20). Probablemente estos cambios se deban a un aumento de la superficie vascular eficaz provocado por la inflamación durante los episodios de peritonitis. La similitud en la respuesta funcional tras un episodio de peritonitis observada con ambas soluciones, indica que la presencia de una infección peritoneal durante el primer año de diálisis limita la reversibilidad de los cambios funcionales que habitualmente ocurren en pacientes sin episodios de peritonitis, que consisten en una convergencia a la mediana de los valores de transporte de pequeños solutos y agua.

En conclusión, hemos observado una gran variabilidad en el transporte de pequeños solutos y agua al inicio en DP en los pacientes tratados con soluciones biocompatibles, aunque esta diversidad es más marcada bajo soluciones convencionales. Existe una tendencia a la normalización de los

parámetros de función peritoneal y aumento de la ultrafiltración durante el primer año en DP, y la aparición de un episodio de peritonitis durante este periodo limita la reversibilidad de estos cambios funcionales en ambos grupos. El uso de soluciones biocompatibles se asocia a menores tasas de ultrafiltración desde el inicio del tratamiento y a un aumento progresivo del transporte de solutos que paradójicamente no se acompaña de disminución de la ultrafiltración.

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Capítulo 7. Histopatología peritoneal en pacientes tratados con soluciones más biocompatibles

6.2.2.1.- "Las soluciones biocompatibles preservan la célula mesotelial y la integridad de la pared vascular. Un estudio caso-control en biopsias humanas"

del Peso G, Jiménez-Heffernan JA, Remón C, Bajo MA, Ossorio M, Fernández-Perpén A, Sánchez-Tomero JA, Cirugeda A, de Sousa E, Sandoval P, López-Cabrera M, Selgas R.

Sometido a 2ª revisión en Peritoneal Dialysis International

Este trabajo responde al objetivo 4

Las soluciones biocompatibles preservan la célula mesotelial y la integridad de la pared vascular. Un estudio caso-control en biopsias humanas.

La exposición crónica a soluciones convencionales se ha relacionado con alteraciones de la función peritoneal en pacientes en DP y con pérdida de células mesoteliales, fibrosis submesotelial, vasculopatía hialinizante y angiogénesis. Estudios i*n vitro*, e*x-vivo* y en modelos animales han demostrado que las soluciones de diálisis biocompatibles atenúan las alteraciones morfológicas.

Objetivo: Comprobar los efectos beneficiosos a nivel morfológico de las soluciones biocompatibles en pacientes en DP.

Métodos: Analizamos la biopsia peritoneal de 23 pacientes tratados con soluciones biocompatibles, y 23 pacientes tratados con soluciones convencionales, comparándolos según el tiempo en DP.

Resultados: Los pacientes tratados con soluciones biocompatibles presentaron mayor preservación total o parcial de la capa mesotelial (56.5% vs. 26.1%, p=0.036), menor vasculopatía hialinizante (4.3% *vs*. 30.4%, p=0.02) y una tendencia a menor presencia de fibrosis peritoneal (47.8% vs. 69.6%, p=0.13) que los que recibían tratamiento con soluciones convencionales. En pacientes sin antecedente de peritontis, se observó significativamente menor prevalencia de fibrosis en el grupo biocompatible (41.7% vs. 77.8%, p=0.04). Se detectó transición epitelio-mesenquimal (TEM) de células mesoteliales en 10 pacientes (22%), sin diferencias significativas entre ambos grupos. En el análisis de

regresión univariante, el uso de soluciones biocompatibles se asoció con integridad mesotelial (p=0.04) y con ausencia de vasculopatía (p=0.04).

Conclusión**:** Este estudio demuestra *in vivo* en biopsias de pacientes en DP que las soluciones biocompatibles son mejor toleradas por el peritoneo a medio-largo plazo que las soluciones convencionales.

Biocompatible dialysis solutions preserve peritoneal mesothelial cell and vessel wall integrity. A case-control study on human biopsies

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Key Words:	Peritoneal Biopsy. , Biocompatible Dialysis Solutions, Mesothelial Cell Integrity. , Hyalinizing Vasculopathy, Submesothelial Fibrosis
Abstract:	Chronic exposure to conventional peritoneal dialysis (PD) solutions has been related to peritoneal function alterations on PD patients, and associated with mesothelial cell loss, submesothelial fibrosis, vasculopathy and angiogenesis. In vitro and and ex-vivo analyses as well as studies with animal models have demonstrated that biocompatible PD solutions attenuate these morphological alterations. Our aim was to confirm the morphological benefits of biocompatible solutions in PD patients. We analyzed biopsies from 23 patients treated with biocompatible solutions (study group, SG), and compared them with a control group (n = 23) treated with conventional solutions (CG), matched for time on PD. Results: 56.5% of SG patients showed total or partial preservation of mesothelial cells monolayer, in contrast with 26.1% of patients in CG (p=0.036). Peritoneal fibrosis was not significantly less frequent in SG patients (47.8%, SG vs. 69.6%, CG; p=0.13). In patients without previous peritonitis, a significantly lower prevalence of fibrosis was present in SG patients (41.7%, SG vs. 77.8%, CG; p=0.04). Hyalinizing Vasculopathy (HV) was significantly lower in the SG group (4.3%, SG vs. 30.4%, CG; p=0.02). Epithelial to mesenchymal transition (EMT) was detected in 10

	patients (22%), but it did not show significantly lower prevalence in SG group. In the univariate regression analysis, the use of biocompatible solutions was associated with mesothelial monolayer integrity (p=0.04) and absence of vasculopathy (p=0.04). Conclusion: The present study offers in vivo demonstration in human biopsies that biocompatible solutions are better tolerated by the peritoneum at medium-and long-term than conventional solutions.

Biocompatible dialysis solutions preserve peritoneal mesothelial cell and vessel wall integrity. A case-control study on human biopsies

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Running title: Better preservation of human peritoneum under biocompatible dialysis solutions

Biocompatible dialysis solutions preserve peritoneal mesothelial cell and vessel wall integrity. A case-control study on human biopsies

Abstract

Chronic exposure to conventional peritoneal dialysis (PD) solutions has been related to peritoneal function alterations on PD patients, and associated with mesothelial cell loss, submesothelial fibrosis, vasculopathy and angiogenesis. *In vitro* and and *ex-vivo* analyses as well as studies with animal models have demonstrated that biocompatible PD solutions attenuate these morphological alterations. Our aim was to confirm the morphological benefits of biocompatible solutions in PD patients.

We analyzed biopsies from 23 patients treated with biocompatible solutions (study group, SG), and compared them with a control group (n = 23) treated with conventional solutions (CG), matched for time on PD.

Results: 56.5% of SG patients showed total or partial preservation of mesothelial cells monolayer, in contrast with 26.1% of patients in CG (p=0.036). Peritoneal fibrosis was not significantly less frequent in SG patients (47.8%, SG vs. 69.6%, CG; p=0.13). In patients without previous peritonitis, a significantly lower prevalence of fibrosis was present in SG patients (41.7%, SG vs. 77.8%, CG; p=0.04). Hyalinizing Vasculopathy (HV) was significantly lower in the SG group (4.3%, SG *vs*. 30.4%, CG; p=0.02). Epithelial to mesenchymal transition (EMT) was detected in 10 patients (22%), but it did not show significantly lower prevalence in SG group. In the univariate regression analysis, the use of biocompatible solutions was associated with mesothelial monolayer integrity (p=0.04) and absence of vasculopathy (p=0.04).

Conclusion: The present study offers *in vivo* demonstration in human biopsies that biocompatible solutions are better tolerated by the peritoneum at medium- and long-term than conventional solutions.

Key words

Peritoneal Biopsy. Biocompatible Dialysis Solutions. Mesothelial Cell Integrity. Hyalinizing Vasculopathy. Submesothelial Fibrosis

Introduction

Peritoneal membrane failure is one of the main limitations for long-term peritoneal dialysis (PD). Chronic exposure to conventional PD solutions and peritoneal inflammation had been described as the main factors associated with peritoneal function alterations in PD patients. Loss of ultrafiltration has been related to peritoneal morphological changes (mesothelial cell loss, submesothelial fibrosis, vasculopathy and angiogenesis), although the exact morpho-functional correlation has not been yet established. It is expected that some peritoneal alterations could be prevented or delayed with more biocompatible PD solutions. Indeed, the use of these newer solutions has been associated with some clinical benefits, such as delayed onset of anuria (1). However, there are conflicting results regarding their favourable effects on peritonitis rates (2) and patient and technical survival, residual renal function and peritoneal membrane function. *In vitro* and *ex vivo* analyses as well as experimental animal studies have demonstrated that biocompatible PD solutions attenuate morphological alterations seen with conventional solutions (3-5). Due to the difficulties of obtaining peritoneal biopsies in clinical practice, we still lack confirmatory *in vivo* morphological evidence of the possible benefits of biocompatible solutions. The histological information regarding peritoneal morphological changes induced by this type of fluids in humans is scarce (6-8). In the present study, we have analyzed biopsies from 23 patients treated for variable periods of time with biocompatible solutions, and we have compared the morphological findings with a control group matched according to time on PD, treated with conventional solutions.

Patients and methods

Study design and patients

A comparative case control matched study of biopsies from 46 stable PD patients was performed; 23 of them were treated with conventional solutions (control group, CG) and 23 with biocompatible solutions (study group, SG). We analysed all available biocompatible peritoneal biopsies collected up to September 2013 in four centres, and we compared them with peritoneal biopsies from patients under conventional solutions in the same time period, matched for time on PD (mean 24.2±18 months in CG vs. 22.7±16 months SG, p=0.8) (**Figure 1**). All SG patients have been using biocompatible solutions since the beginning of PD. None biopsy was taken under inflammatory episode (or at least 30 days apart). In 78% of cases (17 patients in CG, 19 in SG) the peritoneal biopsy was performed during kidney transplant surgery. In the remaining, the biopsy was obtained during incidental abdominal programmed surgery (In CG: three nephrectomies, one omentectomy, one cholecystectomy and one necropsy. In SG: Two catheter removals not related to peritoneal problems, one omentectomy and one polypeptomy).

Clinical characteristics are shown in Table 1. The causes of end-stage renal disease were not significantly different between the two groups (data not shown). Age, gender, diabetes prevalence, and small solute peritoneal transport were similar in both groups (Table 1). Patients in SG used less frequently automated PD than CG patients (43.5%, SG vs. 91%, CS, p=0.001). In control group, five patients suffered eight episodes of peritonitis whereas in the study group 11 patients had 23 episodes.

Peritoneal solute transport and UF were measured during a peritoneal transport kinetic study, using a 2-liter hypertonic glucose exchange during a 4-h dwell time. Peritoneal urea mass transfer coefficient (U-MTAC, ml/min) and creatinine mass transfer coefficient (Cr-MTAC, ml/min) were calculated according to the previously described mathematical model (9). UF was estimated by the negative balance (weighing bags prior infusion and after drainage) during the kinetic study. The peritoneal kinetic studies were performed in the previous months from peritoneal biopsy (a median of 3.4 months, range 0.1-7.6 months in study group, and median 2.8 months, range 0.6-8 months in controls).

Subgroup analysis

We separately performed an analysis according to diabetes and peritonitis episodes, designing three subsets of patients:

- Patients with no peritonitis episodes (n=30) (12 SG, 18 CG)
- Patients with previous peritonitis episodes (n=16) (11 SG, 5 CG)
- Non-diabetic patients (n=41) (20 SG, 21 CG)

PD Solutions

In SG group, seven patients used Physioneal (Baxter Healthcare®), five Balance (Fresenius Medical Care®), six Bicavera (Fresenius Medical Care®) and five GambrosolTrio (Fresenius Medical Care®). In CG, 20 have been using Dianeal (Baxter Healthcare®) and 3 patients Stay-Safe (Fresenius Medical Care®). Icodextrin (Baxter Healthcare®) was used by fourteen patients (61%) in CG and by five patients (22%) in SG (p=0.007).

Peritoneal samples

Parietal peritoneal samples were obtained after opening the anterior abdominal wall. Biopsy collection and processing was done as previously reported (10).

Samples were evaluated by two pathologists without knowledge of clinical data. The morphological parameters analyzed were:

- Mesothelial cell integrity. It was measured using a semiquantitative scale (grade 3, normal cell density; grade 0, complete denudation) as described by *Plum* et al (11).

- Submesothelial thickness. The thickness of the compact zone was measured with a micrometer ocular. The mean of three different measures of representative zones was obtained. When less than 150 μm it was considered normal (Grade 1). If between 150-350 μm it was considered as a moderate thickening (Grade 2). Results greater than 350 μm were regarded as intense thickening (Grade 3). Fibrosis was defined as a submesothelial compact zone measuring more than 150 μm (the highest value recorded for normal subjects)(8).

- Hyalinizing vasculopathy (HV). It was measured using the four grade system described by *Honda* et al (12): grade 0, no abnormalities; grade 1, mild thickening without stenosis of the lumen; grade 2, moderate thickening with partial luminal stenosis; and grade 3, intense thickening with marked stenosis and luminal distortion or complete occlusion.

- *In vivo* evidence of epithelial-to-mesenchymal transition (EMT) (defined by the presence of submesothelial fibroblast-like cells expressing cytokeratin) was also evaluated, as present or absent.

Ethical issues

The procedures were in accordance with the ethical standards of the institutional committee on human experimentation and with the Helsinki Declaration of 1975 (revised in 1983). All patients gave their informed consent.

Statistical analysis

Results are given as means ± SD. A p value <0.05 was considered statistically significant. We used the non-parametric Mann–Whitney *U*-test for comparisons of means between groups and the Fischer exact test for comparisons of proportions. Univariate and multivariate logistic regression analyses were employed to investigate the factors associated with the presence of mesothelial cell integrity, submesothelial fibrosis, HV and EMT on peritoneal biopsy. All statistical analyses were made with SPSS 14.5 (Chicago, IL, USA).

Results

Mesothelial cell integrity

Patients in SG showed significantly greater mesothelial cell preservation score than those in CG (mean values 1.78±1.16 vs. 0.91±0.9, p=0.007). 56.5% of SG patients showed total or partial preservation of mesothelial cells (score 2-3), in contrast with 26.1% of patients in CG (p=0.036) (**Figure 2**).

To rule out a negative effect of prior inflammation on mesothelial preservation, patients without previous peritonitis were examined. These patients under biocompatible solutions showed more frequently scores 2-3 than those from control group (67% SG vs. 28% CG, p=0.035). However, patients with peritonitis antecedents (n=16; 11 SG and 5 CG) showed non-significant differences (45.5% SG vs. 20% CG, p=0.33), probably due to the low number of patients.

Non-diabetic patients, as they represent the majority of the series, showed also significantly better preservation of mesothelial layer among SG (60%) than in CG (29%)(p=0.043).

Submesothelial thickness

Peritoneal fibrosis was less frequent in biocompatible solution patients, although it did not reach statistical significance (47.8%, SG vs. 69.6%, CG, p=0.13). The average score confirmed the lack of significant differences (1.09±1.1, SG vs. 1.7±0.97, CG, p=0.06). Extreme values of submesothelial thickness (>350 μm) were less common in SG (8.7% vs. 17.4%), but again differences were not significant.

However, when patients with previous peritonitis were excluded, a significant lower prevalence of fibrosis was present in SG patients (41.7%, SG vs. 77.8%, CG) (p=0.04). In contrast, these differences did not appear between both groups

in patients with previous peritonitis (p=0.59) and in non-diabetic patients (p=0.28).

Hyalinizing vasculopathy (HV) (**Figure 3**)

The prevalence of HV was significantly lower in the group treated with biocompatible solutions (4.3%, SG *vs*. 30.4%, CG, p=0.02). The average score was also lower in SG (0.09±0.41, SG vs. 0.48±0.8, CG, p=0.05). When patients with peritonitis were removed, none patient from SG showed HV, in contrast with 33.3% from CG (p=0.025). Patients with prior peritonitis showed similar HV prevalence with both type of solutions (p=0.54).

Among non-diabetic patients, HV also showed a lower prevalence with biocompatible solutions (5%, SG *vs*. 28.6%, CG, p=0.045).

Epithelial-to-Mesenchymal transition (Submesothelial cytokeratin staining)

EMT was detected only in 10 patients (22%), with a lower prevalence in SG group, but it did not reach statistical significance probably due to the low number of patients (13%, SG *vs*. 30.4%, CG, p=0.15). Interestingly, when EMT was present, mesothelial layer integrity (scores 2-3) never appeared (for both solutions). The incidence of early EMT (less than two years on PD) did not reach statistical significant differences between the two groups (13.3 % SG showed EMT in contrast with 33.3% from CG, p=0.19).

We also observed a higher prevalence of EMT, although no statistically significant, in patients with previous peritonitis in both groups (data not shown). Among non-diabetics, a lower but also non-significant prevalence of EMT in SG was present (p=0.3).

Morpho-functional discrepancy

Although SG patients showed clearly better morphology scores than CG, UF capacity was significantly lower in that group, with no other peritoneal transport parameters showing differences (Table 1).

Logistic regression analysis

In the univariate regression analysis, the use of biocompatible solutions was associated with the presence of mesothelial cell integrity (odds ratio 3.68; confidence interval: 1.06-12.7, p=0.04), and persisted significant after adjusting to age, time on PD, diabetes, and peritoneal transport parameters (Cr-MTC, UF capacity) in the multivariate analysis. The significance was lost when accumulated glucose load and accumulated days of peritonitis were added to the model.

In the univariate analysis, the use of conventional solutions was the only factor predicting the presence of HV (odds ratio 9.62; confidence interval: 1.07-86.17, p=0.04), also after adjusting for age, time on PD, peritonitis, and diabetes in the multivariate analysis. The significance was lost when adjusted by peritoneal functional parameters and glucose load.

Discussion

This study represents the first evidence *in vivo* of a benefit of biocompatible solutions on peritoneal morphology in a short series of non-selected PD patients. Peritoneal biopsies from patients on PD with biocompatible solutions showed better mesothelial cell monolayer preservation and lower prevalence of hyalinizing vasculopathy when compared biopsies from patients treated with conventional solutions during a similar period of time. In addition, when patients with prior peritonitis episodes were excluded, we also found a less induction of submesothelial fibrosis with biocompatible PD fluids. Improvements on these morphological variables (mesothelial integrity, fibrosis and vasculopathy) demonstrate a better peritoneal protection by the new PD solutions.

These *in vivo* findings on PD patients confirm previous *in vitro* and *ex vivo* studies and experiments in animal models showing that biocompatible solutions result in a better viability of peritoneal membrane (4-6, 13-16) , particularly of mesothelial cells. *Bajo* et al (4) described that *in vitro* exposure of mesothelial cells to conventional fluids resulted into a non-epitheloid shape and down-regulation of E-cadherin, indicatives of EMT, and in a strong induction of vascular endothelial growth factor (VEGF) expression. In contrast, cells *in vitro* exposed to low-GDP solution did not develop these phenotype changes. They also observed *ex vivo* that the prevalence of non-epitheloid phenotype in the conventional group was significantly higher with increasing PD duration but, in contrast, mesothelial cells from PD effluent of patients treated with low-GDP fluids had fewer signs of EMT. *Fernández-Perpén* et al (16) also showed that *ex vivo* mesothelial cells grown from effluent of patients treated with

bicarbonate/low-GDP fluid showed a trend to acquire an epithelial phenotype, with lower production of pro-inflammatory cytokines and chemokines than those from patients treated with a lactate-buffered conventional PD solution. These two studies were released by the same group of investigators and are complementary, but very consistent. Other studies describing the anatomical alterations with biocompatible solutions are scant. In 2005, *Do* et al.(6) reported less *ex vivo* EMT induction and faster remesothelialization with low-GDP fluids in a randomized prospective controlled study in 60 PD patients, when compared with high-GDP solutions. *Ayuzawa* et al (7) showed in patients with more than three years on PD, a lower induction of peritoneal fibrosis and vasculopathy with the use of hybrid therapy, combining PD with biocompatible solutions and hemodialysis. More recently, *Kawanishi* et al (8) have described in 12 patients treated with low-GDPs neutral solutions, less induction of peritoneal fibrosis, vascular sclerosis and advanced glycation end-product accumulation, but an increased blood capillary density, when compared with 12 patients treated with acidic high-GDPs solutions. However, the low number of patients in such study and the fact that peritoneal specimens were obtained during catheter removal, probably due to peritoneal membrane problems, makes necessary new series.

Animal studies also have shown attenuation on peritoneal fibrosis and vascularization with low glucose degradation products (GDP) related to conventional solutions. *Zareie* et al (17) demonstrated reduced mesothelial damage, fibrosis and angiogenesis after chronic peritoneal exposure to amino acid-based peritoneal dialysis fluid (PDF) compared to glucose-containing PDF in rats. *Mortier* et al (18) reported that low-GDP bicarbonate/lactate-buffered and amino acid-based PDF preserved better peritoneal integrity in rats than

conventional PDF. *Garosi* et al (19) also observed less mesothelial cell damage, lower submesothelial fibrosis and absence of vascular alterations in rabbits dialyzed with amino acid-based PDF.

Another interesting observation from our study is that these anatomical benefits may be neutralized by repeated episodes of peritonitis. All the analysis performed removing these patients from the series confirmed the previous differences (mesothelial and vasculopathy scores lower among SG patients) and showed significant differences in fibrosis score. It seems plausible that peritoneal infections causing inflammation modify the peritoneal response to PD fluids, masking their potential benefits.

EMT of mesothelial cells has emerged as a pathogenic mechanism leading to structural changes and peritoneal dysfunction. Although not the only mechanism leading to myofibroblastic activation, EMT is an important contributor to peritoneal damage (20). In response to conventional solutions or peritonitis a subset of injured mesothelial cells transform into myofibroblasts capable of producing fibrosis and angiogenesis. Part of the protective effect of biocompatible solutions depends on its greater mesothelial preservation and therefore the attenuation of EMT. Our present study confirms, although with a limited significance, differences in EMT that was more prevalent (three times) among conventional solution patients. The causes of this limitation may lead in the low number of patients, the low sensitivity of EMT markers in tissue and the potential confounding factor that represents peritonitis.

Other **limitations** of the study concern the matching procedure. Significantly, control group is younger than study group and uses more frequently automated PD; these two factors are intrinsically associated for patient reasons, since

younger patients use more often APD. The case-control design according to time on PD permits the comparisons at equivalent PD duration, a well recognized factor implicated in the morphological alterations of peritoneal membrane at mid-long term.

The main strength of our study is the large number of peritoneal biopsy samples collected, despite the difficulties in obtaining them.

In **conclusion**, the present study offers *in vivo* demonstration in human biopsies that biocompatible solutions are better tolerated by the peritoneum at medium-and long-term than conventional solutions. In consequence, we suggest that peritoneal dialysis must be currently offered with biocompatible solutions, with the expectancy of attenuating or delaying local complications at long-term.

Disclosures

Nothing to declare

Table 1. Patient characteristics at the time of peritoneal biopsy

	CONTROL GROUP (n=23)	STUDY GROUP (n=23)	P
Age (years)	43.8±14	51.5±14	ns
Male gender	12 (52%)	15 (65%)	ns
Time on PD (months)	24.2±18	22.7±16	ns
Automated PD	21 (91%)	10 (43.5%)	0.001
Accumulated glucose load (Kilograms)	139.2+151	101.08+110	ns
Diabetes	2 (8.7%)	3 (13%)	ns
Peritonitis antecedent	5 (21.7%)	11 (48%)	ns
Mean number of peritonitis	1.6±1.3	2.09±1.44	ns
Accumulated days of peritonitis	2.8±2	5.2±4	ns
Urea-MTC (ml/min)	21.5±7	23.5±4	ns
Cr-MTC (ml/min)	8.9±4	9.3±2.5	ns
UF capacity (ml/4h)	849±257	666±261	0.041
Residual renal function (ml/min)	3.22±3.46	5.08±3.5	ns

MTC= mass transfer coefficient. UF= ultrafiltration

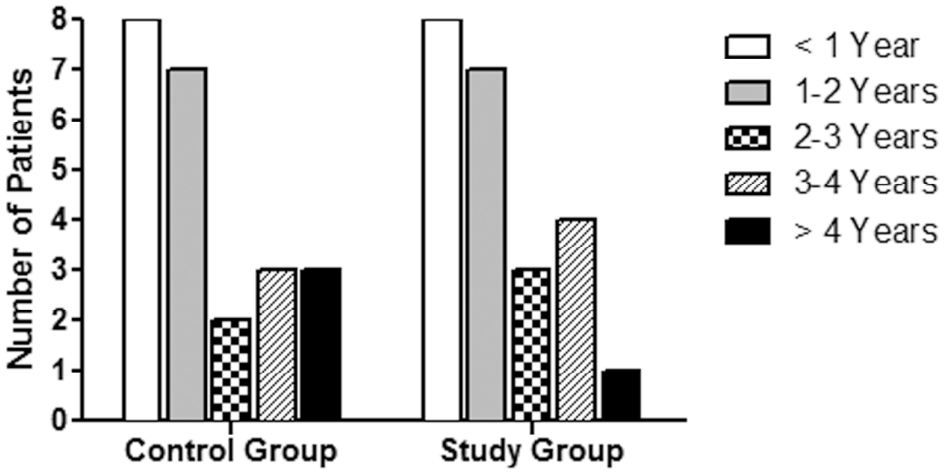
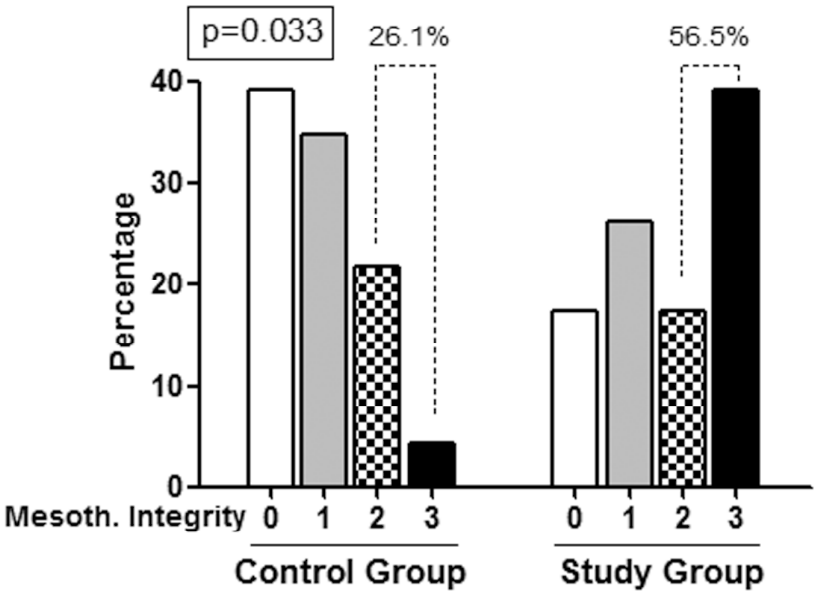


Figure 1


p=0.033
26.1%
56.5%
40
30
20
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Percentage
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0
1
2
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1
2
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Control Group
Study Group

Figure 2

Figure Legend

Figure 1. Distribution of patients according to time on PD, expressed in years, in both groups

Figure 2. Frequency of patients with different mesothelial preservation score in the two groups. Patients under biocompatible solutions presented much more frequently high scores of mesothelial layer preservation

Figure 3. Peritoneal biopsies from patients receiving biocompatible solutions (a,c,e) showed better mesothelial cell preservation, less submesothelial thickness and hyalinizing vasculopathy when compared to patients treated with conventional fluids (b,d,f). Grade 1 hyalinizing vasculopathy lesions are seen on image b (arrow). A clear contrast among mesothelial cell preservation is evident on all images (a,b,c,d: hematoxylin and eosin, x200). Immunohistochemistry for cytokeratins reveals a modified, superficial mesothelial cell (arrow) that contrasts with the well preserved layer seen on a biocompatible patient (e,f: immunoperoxidase, x400).

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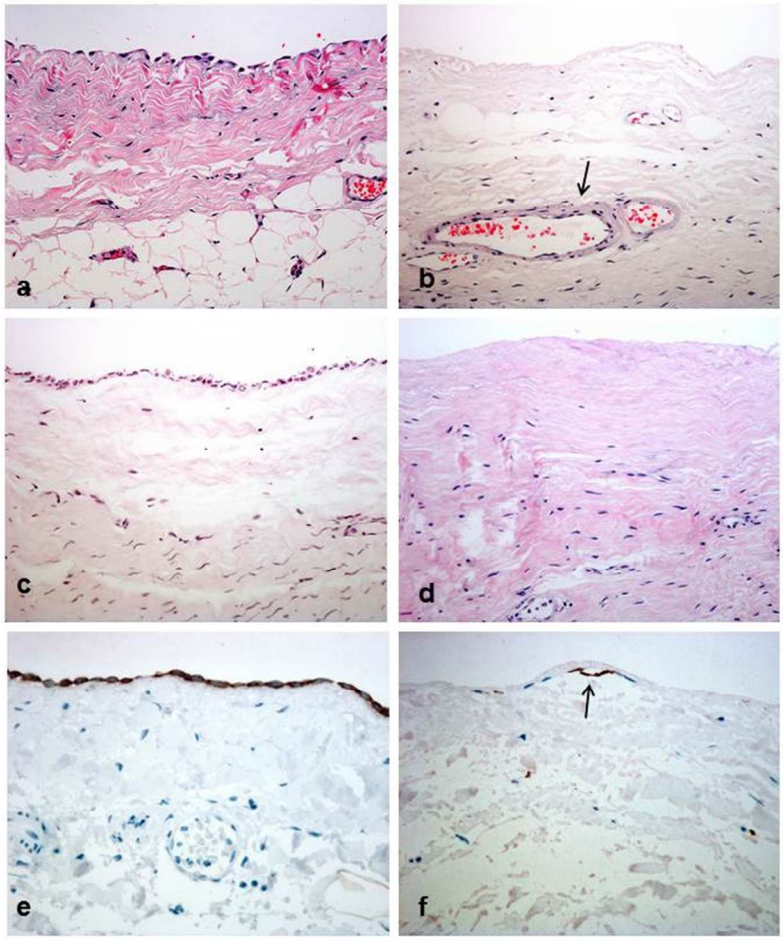

Peritoneal biopsies from patients receiving biocompatible solutions (a,c,e) showed better mesothelial cell preservation, less submesothelial thickness and hyalinizing vasculopathy when compared to patients treated with conventional fluids (b,d,f). Grade 1 hyalinizing vasculopathy lesions are seen on image b (arrow). A clear contrast among mesothelial cell preservation is evident on all images (a,b,c,d: hematoxylin and eosin, x200). Immunohistochemistry for cytokeratins reveals a modified, superficial mesothelial cell (arrow) that contrasts with the well preserved layer seen on a biocompatible patient (e,f: immunoperoxidase, x400).
190x275mm (96 x 96 DPI)

7.- DISCUSIÓN

Esta tesis nació de la necesidad de conocer el verdadero sustrato morfológico y los mecanismos fisiopatológicos implicados en las alteraciones funcionales peritoneales que observamos en los pacientes tratados con diálisis peritoneal en la práctica clínica diaria. A lo largo de varios años de estudio, hemos tratado de dar respuesta a algunas preguntas, intentando acercarnos lo más posible a lo que verdaderamente sucede en el peritoneo de nuestros pacientes, conscientes de que los estudios de que disponemos para analizar la función peritoneal pueden no reflejar la auténtica realidad. Para ello, hemos analizado cómo se comporta funcionalmente el peritoneo de nuestros pacientes desde su inicio, y paralelamente estudiamos los cambios morfológicos que se producen. Esta tesis recoge la experiencia de más de 30 años en una amplia serie de pacientes tratados con esta técnica con soluciones convencionales. En la última etapa, tras la introducción de las soluciones más biocompatibles, hemos analizado la repercusión funcional y morfológica que pueden tener éstas en la membrana peritoneal.

En definitiva, a lo largo de todos estos años hemos investigado cómo es la interacción entre el tiempo en DP y el tejido peritoneal, cuando éste interactúa con distintas soluciones de diálisis.

La diálisis peritoneal a corto plazo con soluciones convencionales

Con el fin de conocer cómo es la respuesta inicial del peritoneo a la DP, y para estudiar los mecanismos etiopatogénicos que originan las lesiones descritas en pacientes con largas estancias en DP, analizamos cuáles eran los cambios morfo-funcionales peritoneales al comienzo de la terapia. Por ello, el

primer objetivo de la tesis fue conocer cómo eran las características de la membrana peritoneal antes de interactuar con las soluciones de diálisis, es decir, cuando sólo intervienen factores derivados de las características propias de cada paciente. En el capítulo 1 demostramos que el comportamiento funcional del peritoneo en las primeras semanas de tratamiento es enormemente variable, hecho que ya ha sido descrito por otros grupos (42). Como se observa en dicho capítulo, existe un grupo de pacientes que ya presentan déficit en la capacidad de UF o alto transporte de pequeños solutos al inicio de DP. El **AT inherente** fue hace años asociado a una menor supervivencia, tanto de la técnica como del paciente (6), pero estudios más recientes, incluido uno realizado por nuestro grupo (46) han demostrado que ésto sólo es cierto si el paciente tiene una elevada comorbilidad y no es adecuadamente tratado con las opciones terapéuticas que han ido surgiendo en los últimos años (DP automática e icodextrina)(47). En el capítulo 5 hemos encontrado que la medición de la función peritoneal en las primeras semanas de tratamiento es poco representativa de su evolución posterior, siendo la cinética peritoneal realizada al año de diálisis la que mejor nos indica el pronóstico funcional peritoneal. Otro hecho importante es que, a diferencia de lo descrito en pacientes con largas estancias en la técnica (53), al inicio del tratamiento con DP con soluciones convencionales no siempre existe una relación inversa entre el transporte de pequeños solutos y agua. La correlación entre baja capacidad de UF y AT inherente es mucho más débil que con el AT adquirido, de forma que no todos los pacientes con AT tienen fallo de UF y viceversa. Esta pérdida de concordancia sugiere que el fallo UF inherente no es solo derivado de una pérdida acelerada de gradiente osmótico secundario al

AT de pequeños solutos, sino que puede haber otros factores quizá relacionados con una alteración de otros componentes de la membrana, como es la matriz extracelular. En este sentido, el hallazgo de matriz extracelular de aspecto homogéneo y escasamente celular, presente en los pacientes en DP a diferencia de lo observado en otros modelos de fibrosis (84), sugiere la posibilidad de que existan cambios cualitativos en el intersticio peritoneal aún no conocidos que justifiquen la disociación observada entre los transportes de pequeños solutos y agua.

El AT inherente es una alteración reversible durante los primeros años en DP, siempre y cuando no existan peritonitis ni alta exposición a glucosa durante ese periodo. La reversibilidad del AT inicial sugiere que probablemente factores relacionados con la homeostasis vascular, en concreto vasodilatación de la microvasculatura peritoneal, pueden ser responsables de este fenómeno. El estudio realizado en el capítulo 4, donde no hemos encontrado asociación entre AT y angiogénesis en pacientes con menos de dos años en DP, apoya esta idea.

Tras el conocimiento de la diversidad funcional peritoneal basal, nos propusimos explorar cómo es el peritoneo de los pacientes a su llegada a DP, y evaluar el papel que pudiera ejercer la **uremia** en las alteraciones anatómicas peritoneales. Para ello, lo primero que debíamos conocer es cómo es la membrana peritoneal de los sujetos sanos y de los pacientes urémicos que no reciben tratamiento con DP, objeto de estudio en el capítulo 2. En nuestra serie de biopsias peritoneales de pacientes en DP, hemos observado que las lesiones morfológicas más frecuentemente presentes son la pérdida de la monocapa mesotelial, la fibrosis submesotelial y la vasculopatía hialinizante,

similar a lo descrito por otros grupos (32). Los sujetos sanos no presentan ninguna de estas alteraciones, mientras que los pacientes urémicos sin diálisis (pacientes en situación de prediálisis o en tratamiento con hemodiálisis) tienen mínimas lesiones de fibrosis y no muestran VH. Estos hallazgos están en concordancia con lo referido por varios autores (31,32,33), que encuentran ausencia o lesiones leves de fibrosis y VH antes de diálisis. Una vez iniciada la DP, el estudio realizado por nuestro grupo en el capítulo 2, confirma el hecho de que los factores relacionados con la propia DP, y no la uremia, son los principales determinantes de los hallazgos morfológicos encontrados en nuestros pacientes. En él mostramos además que la presencia de diabetes tiene poca repercusión en la histopatología peritoneal una vez comenzada la técnica, hallazgo importante a la hora de interpretar los hallazgos histológicos, dado que existe un elevado número de pacientes diabéticos que son tratados con DP en nuestro medio.

En base a los estudios realizados en los capítulos 3 y 5, y también observado previamente por otros autores (54), hemos demostrado que durante los dos primeros años de tratamiento con DP la evolución del transporte de pequeños solutos y agua difiere según el punto de partida, existiendo una tendencia general a la normalización en ambos. Pero esto sólo es cierto en ausencia durante ese periodo de agresiones peritoneales locales, como episodios reiterados o graves de peritonitis, en los que esta tendencia se invierte. Posteriormente, se observa una estabilidad hasta el 3º-4º año en DP, cuando se inicia un incremento progresivo del transporte de pequeños solutos y un descenso de la capacidad de UF en un elevado número de pacientes, fenómeno confirmado en otras series (53).

Cuando exploramos el sustrato anatómico del AT en los primeros años de DP, en los capítulos 2 y 4 observamos que la principal lesión asociada era la presencia de **transición epitelio-mesenquimal (TEM)** de la célula mesotelial peritoneal (expresión de marcadores mesoteliales en miofibroblastos submesoteliales), también denominada transdiferenciación. Previamente, como se describe en el capítulo 2, los análisis *in vitro* y *ex vivo* realizados por nuestro grupo y confirmados en biopsias peritoneales de pacientes en DP, habían demostrado la existencia de TEM mesotelial en peritoneo. A diferencia de los pacientes en DP, no la observamos en sujetos sanos ni en pacientes urémicos sin DP. En base a estudios experimentales que implican a la TEM en la génesis de fibrosis, es razonable pensar que juegue un papel fundamental en la fibrogénesis de los pacientes en DP. De hecho, en nuestra serie de biopsias, los pacientes con fibrosis son los que tenían mayor prevalencia de TEM. Además, un estudio previo de nuestro grupo mostró la presencia de esta misma lesión en pacientes con **peritonitis** aguda no urémicos (84). Esto podría ser el nexo de unión entre la inflamación local y el desarrollo de fibrosis peritoneal, y explicaría parte de los efectos deletéreos de las peritonitis sobre el transporte de pequeños solutos y agua que hemos observado (capítulos 3 y 5). El hallazgo de niveles locales elevados de TGFβ, conocido inductor de TEM (85,86), en pacientes en DP con peritonitis, apoya esta teoría (87).

<u>La diálisis peritoneal a medio-largo plazo con soluciones convencionales</u>

Como ya hemos mencionado, la limitación más importante de la DP es su viabilidad a largo plazo. Sin embargo, existen muy pocas series con un elevado número de pacientes que hayan analizado a largo plazo la función

peritoneal (32), siendo todavía más escasos los estudios que estudian la histopatología peritoneal correspondiente (81,82). El hecho diferencial de nuestra serie de biopsias peritoneales es no estar centrada en pacientes con fracaso de la técnica.

En concordancia con lo referido por otros grupos (52, 57), hemos observado una relación directa entre el AT adquirido y el antecedente de peritonitis o el abuso de soluciones con glucosa (capítulos 3 y 5). En el capítulo 5 encontramos que el **abuso de soluciones ricas en glucosa** provoca AT adquirido, independientemente del tipo de transporte inicial. Si bien no fue analizada la exposición a glucosa en nuestra serie de biopsias en pacientes con soluciones convencionales, hay estudios en animales que han encontrado alteraciones anatómicas directamente derivadas de su abuso (88).

El verdadero mecanismo patogénico del AT adquirido en los pacientes en DP no es del todo conocido. Desde el punto de vista histológico, el aumento del transporte de pequeños solutos ha sido relacionado con **angiogénesis** peritoneal. A diferencia de lo descrito clásicamente (30), nuestro grupo (en el capítulo 4) y otros autores (32, 59) hemos observado que la presencia de angiogénesis no se asocia constantemente con el tiempo en DP. Deben por tanto postularse otros mecanismos patogénicos en el AT adquirido, como un aumento de la permeabilidad vascular peritoneal o alteraciones cualitativas de la matriz extracelular. En este sentido, en el capítulo 4 se demuestra que los pacientes con AT presentan mayor expresión de **VEGF** peritoneal, sin incremento en la densidad vascular. Esto apoyaría la idea de que el mecanismo patogénico implicado en el AT fuera el aumento de permeabilidad en la microvascularización peritoneal, y no necesariamente la existencia de

angiogénesis secundaria al aumento de VEGF. La mayor expresión de VEGF observada en pacientes con AT coincidiendo con mayor presencia de TEM mesotelial, sugiere que este proceso sería la principal fuente de VEGF. Estudios *in vitro* y *ex vivo* de nuestro grupo (89) ya habían demostrado que las células mesoteliales transdiferenciadas son productoras de enormes cantidades de VEGF.

El hallazgo de la TEM mesotelial (capítulo 2) cambió nuestra visión de la etiopatogenia del AT peritoneal de pequeños solutos en pacientes en DP. La TEM en otros tejidos ha sido implicada en la génesis de fibrosis y angiogénesis (90), ambos hallazgos encontrados en pacientes con largas estancias en DP y AT adquirido. Esta lesión precedería al desarrollo de las lesiones de fibrosis submesotelial y proliferación vascular observadas en estos pacientes. Esta misma secuencia de fenómenos ha sido demostrada también en modelos animales (85). La confirmación de la TEM mesotelial como agente iniciador de las lesiones de AT adquirido ofrece la posibilidad de investigar terapias que frenen el proceso en fases aún reversibles.

La diálisis peritoneal con soluciones más biocompatibles

Según se muestra en el capítulo 6, el comportamiento funcional del peritoneo en contacto con soluciones más biocompatibles muestra algunas diferencias respecto al observado con las soluciones convencionales. Una fundamental es la pérdida de la relación inversa del transporte de pequeños solutos y agua en pacientes con medias-largas estancias en DP, de forma que el aumento progresivo de este transporte no se acompaña siempre de fallo de UF. Bajo soluciones convencionales, con el tiempo en DP suele haber un incremento del transporte de pequeños solutos con pérdida rápida del

gradiente osmótico. Este fenómeno induce una disminución de la capacidad de UF por retorno del ultrafiltrado al capilar peritoneal. Pero ésta es una interpretación algo simplista. Como hemos mostrado en el capítulo 6, bajo el tratamiento con soluciones más biocompatibles observamos un aumento del transporte de solutos sin disminución del de agua. Pero dado que hay preservación del gradiente osmótico de glucosa, no se puede ya invocar un incremento bidireccional del transporte de solutos. Estas diferencias podrían ser en parte explicadas por los hallazgos histopatológicos comentados en el capítulo 7. El primero y más importante es la mayor preservación de la integridad mesotelial en pacientes en tratamiento con soluciones más biocompatibles. Nuestro grupo propone que la pérdida de la monocapa mesotelial es el punto de partida de la lesión peritoneal. Este hecho, unido a la menor inducción de fibrosis submesotelial por estas soluciones (siempre que no existan episodios de peritonitis), sugiere que pueden provocar menor conversión miofibroblástica de la célula mesotelial. La menor inducción de TEM mesotelial con estas soluciones ha sido demostrada en estudios *in-vitro* y *ex-vivo* por nuestro grupo (66,67) y por otros autores (73). Esta menor presencia se debería acompañar de una menor concentración de VEGF, debido a que las células mesoteliales transformadas son productoras de 100-1000 veces más VEGF a nivel local que las no transformadas (capítulo 4). El VEGF, a través de su potente poder vasodilatador debe incrementar notablemente el paso bidireccional interendotelial.

Otro hallazgo relevante del capítulo 7 es la menor presencia y severidad de vasculopatía hialinizante en pacientes con soluciones más biocompatibles. Esta lesión se ha atribuido a una elevada concentración de glucosa a ambos

lados de la pared vascular, pero su verdadera etiopatogenia no está aún bien definida. En el capítulo 2 hemos observado que los pacientes en DP muestran VH en los vasos peritoneales pero no en los extraperitoneales de serosas similares (pleura, pericardio…), lo que claramente sugiere una relación etiopatogénica con la presencia y composición de los líquidos de diálisis. Una posible explicación del desarrollo de VH sería el trasiego interendotelial de proteínas que presentan estos pacientes. Éste provocaría un acúmulo progresivo de material hialino en la pared de los vasos peritoneales, de forma similar a lo descrito en la hialinización de la pared vascular de los pacientes con hipertensión arterial. Otro fenómeno que podría contribuir al desarrollo de VH es la transición endotelio-mesenquimal (transformación de una célula endotelial en célula fibroblástica), ya descrita en pacientes con otros procesos fibrosantes (fibrosis pulmonar o esclerosis sistémica). Este proceso estaría inducido por la propia glucosa y PDGs de las soluciones de diálisis y favorecería una mayor trasudación proteica hacia el subendotelio. Sin embargo, en pacientes en DP esta lesión no ha sido todavía objeto de estudio; por ello, el conocimiento de los factores implicados en la patogenia de la hialinización de la pared vascular en DP es un campo aún por explorar. Las nuevas líneas de investigación deben ir encaminadas en ese sentido, para tratar de explicar la menor prevalencia y severidad de VH en pacientes tratados con las soluciones más biocompatibles.

Otro elemento que debemos tener en cuenta para intentar explicar las diferencias funcionales observadas en pacientes tratados con distintas soluciones es la composición de la matriz extracelular. Pero el intersticio peritoneal es a día de hoy la parte más desconocida de la membrana, de ahí que los futuros estudios del peritoneo deberían incluir su análisis.

Esta tesis tiene algunas **limitaciones**, fundamentalmente derivadas de los métodos empleados para la medición de la función y la morfología peritoneal. Desde el punto de vista funcional, no todos los estudios cinéticos realizados en pacientes tratados con soluciones convencionales se han podido realizar según lo recomendado por la Sociedad Internacional de Diálisis Peritoneal (83) utilizando glucosa hipertónica. Esto es debido a que algunos de ellos son anteriores a la recomendación de estandarización de las mediciones. No obstante, salvo este hecho, el método utilizado a lo largo de todos estos años ha sido siempre el mismo, lo que permite homogeneizar la interpretación de los resultados.

Por lo que respecta al análisis morfológico, la limitación más importante es la dificultad en la obtención de un amplio número de muestras, debido a la invasividad de la técnica, siendo conscientes de que la situación ideal sería una recogida seriada para conocer de forma más fiable la fisiopatología peritoneal y las verdaderas asociaciones anatomo-funcionales a lo largo del tiempo en DP.

Las principales **fortalezas** de esta tesis son el alto número de pacientes analizados en los estudios funcionales a lo largo de muchos años, en un grupo de trabajo que tiene criterios unificados. Además, como ya hemos comentado, uno de sus aspectos fundamentales es que las biopsias peritoneales han sido obtenidas en pacientes sin problemas de la membrana peritoneal, a diferencia de la mayoría de grupos que han analizado la histopatología peritoneal en pacientes en situaciones adversas.

8.- CONCLUSIONES

Relacionadas con las soluciones convencionales:

1.- Su uso se asocia con una gran variabilidad en el transporte de solutos y agua al inicio del tratamiento con diálisis peritoneal. Ambos transportes suelen estar disociados en esta fase.

2.- El transporte de solutos y agua tiende a homogeneizarse durante los dos primeros años en la técnica, salvo que existan episodios graves o frecuentes de peritonitis o abuso de soluciones ricas en glucosa.

3.- Las lesiones morfológicas más frecuentemente asociadas con el tiempo en diálisis son la pérdida de la monocapa mesotelial, la fibrosis submesotelial y la vasculopatía hialinizante. La presencia de angiogénesis en la membrana peritoneal no está presente de forma constante.

4.- La uremia y la diabetes contribuyen de forma poco significativa a las lesiones de fibrosis y vasculopatía hialinizante observadas inicialmente en los pacientes en diálisis peritoneal.

5.- La lesión morfológica más precoz asociada al alto transporte de solutos es la transición epitelio-mesenquimal de la célula mesotelial.

6.- Un alto porcentaje de pacientes muestra con el tiempo en diálisis un aumento del transporte de pequeños solutos, asociado con un descenso de la capacidad de ultrafiltración.

7.- Las peritonitis y el abuso de soluciones con glucosa son los principales determinantes del deterioro anatómico y funcional observado en los pacientes en diálisis peritoneal.

8.- La transición epitelio-mesenquimal mesotelial participa en la génesis del alto transporte de pequeños solutos, induciendo fibrosis e hiperpermeabilidad

peritoneal (a través de un aumento del factor de crecimiento del endotelio vascular).

Relacionadas con las soluciones más biocompatibles:

9.- Funcionalmente, los pacientes tratados con soluciones biocompatibles tienen un comportamiento diferente al observado en los tratados con soluciones convencionales. No muestran la relación inversa entre el transporte de solutos y agua, lo que sugiere que estas soluciones provocan cambios cualitativos diferentes en la membrana peritoneal.

10.- El transporte de solutos y agua también tiende a homogeneizarse durante los dos primeros años en la técnica, salvo que existan episodios graves o frecuentes de peritonitis o abuso de soluciones ricas en glucosa. Esto implica que ambos fenómenos son determinantes de la evolución de la membrana, independientemente del tipo de solución utilizada.

11.- La membrana peritoneal muestra, desde el punto de vista anatómico, una mejor tolerancia a las soluciones biocompatibles, representada por una mayor preservación de la monocapa mesotelial, menor inducción de fibrosis, y menor presencia y severidad de vasculopatía hialinizante.

Conclusión final

Nuestro conocimiento de la función peritoneal ha estado basado en la interpretación de lo observado bajo soluciones convencionales, tanto al inicio de DP como durante su seguimiento. Las soluciones más biocompatibles cambian los paradigmas y cuestionan esta interpretación. La observación de

los fenómenos funcionales peritoneales con el apoyo de un adecuado conocimiento histológico nos permitirá asegurar dichas interpretaciones.

Los resultados positivos que muestran muchos pacientes tratados con soluciones más biocompatibles en la biopsia peritoneal, sugiere de forma evidente una mayor preservación de la integridad de la membrana.

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